# SURVEILLANCE OF STREPTOCOCCAL INFECTION IN A SOUTH INDIAN COMMUNITY MEDICAL PROGRAMME

FINAL REPORT

A STUDY SUPPORTED BY
THE NETHERLANDS MINISTRY OF DEVELOPMENT COOPERATION
1975 - 1978

## CONTROL OF RHEUMATIC FEVER AND RHEUMATIC HEART DISEASE IN RURAL SCHOOL CHILDREN IN SOUTHERN INDIA

Principal Investigator :

**Dr. GRACE KOSHI,** B.Sc., M.B.B.S., D.C.P., M.D.
Professor of Microbiology,
Christian Medical College Hospital,
Vellore-632 004, Tamil Nadu, India.

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FINAL REPORT
OF
THE SUCCESS OF SURVEILLANCE AND PROPHYLAXIS IN THE
CONTROL OF RHEUMATIC FEVER AND RHEUMATIC HEART DISEASE
IN RURAL SCHOOL CHILDREN IN SOUTH INDIA.

Dr. V. Benjamin
8, Clive Road
Cent[illegible]
[illegible]

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**Prof. GRACE KOSHI,**
Head, Department of Microbiology,
Christian Medical College Hospital,
Vellore-632 004, Tamil Nadu, India.

Dr. V. Benjamin
8, Clive Road
Cook Town
BANGALORE 560 008

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# Surveillance of Streptococcal Infection in a South Indian Community Medical Programme

GRANTING AGENCY : NETHERLANDS MINISTRY OF DEVELOPMENT CO-OPERATION

GRANTEE AGENCY : CHRISTIAN MEDICAL COLLEGE HOSPITAL VELLORE 632 004, SOUTH INDIA

PRINCIPAL INVESTIGATOR : GRACE KOSHI, B.Sc., M.B.B.S., D.C.P., M.D.,
Professor & Head
Department of Microbiology
C. M. C. Hospital, VELLORE-632 004

CO-INVESTIGATORS : (i) V. BENJAMIN, M.P.H , M.R.C.P. (E), F.R.F.P.S. (G).
Professor & Head
Department of Community Health
C. M. C. Hospital, VELLORE-632 004

(ii) GEORGE CHERIAN, M.D., D.M., F.A.C.P., F.R. C.P., F.A.M.S.
Professor & Head
Department of Cardiology
C. M. C. Hospital, VELLORE-632 004

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2. Biostatistics
3. Clinical Pathology
4. Clinical Biochemistry

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# PROJECT STAFF

| Category | Name |
|---|---|
| Medical Officers | Dr. Uma Krishnamurthy |
| | Dr. Usha Puliyel |
| | Dr. C. Chandran |
| | Dr. Sabitha Loganathan |
| Research Assistants | Mr. C. P. Thangavelu |
| | Mr. K. N. Brahmadathan |
| Microbiologists | Mrs. Rajeswari Pandian |
| | Mrs. Sosamma John |
| Technicians | Mr. M. Mahimaidoss |
| | Mr. M. Arokianathan |
| | Mr. A. Kamalakaran |
| Social Worker | Miss Vimala Padmini |
| Field Workers | Mrs. S. Kanthimathi |
| | Mrs. Jane Swami Pillai |
| | Miss B. Vasantha Bai |
| | Mrs. Martha Devadoss |
| Clerical Staff | Mr. T. Parthiban |
| | Mr. Samuel Jabakumar |
| | Mr. G. Venkatesan |
| Attender | Mr. S. Natarajan |
| Public Health Nurse | Miss Lalitha David |

## Preface

Streptococcal infections are a world-wide problem encountered in all countries and India is no exception. Though rheumatic fever (RF) has continued to decline and debilitating heart disease is less common in the affluent countries, they continue to be common in many tropical countries as well as in the slums of affluent countries. The dreaded complications of RF and rheumatic heart disease (RHD) are prevalent in different parts of India and other South-east Asian countries, Africa and S. America and have been detected wherever they have been looked for. They account for one third of all cardiac admissions in all major hospitals in India. However, data based on hospital admissions does not always reflect what is actually found in the general population.

The impetus for initiating an epidemiological study on streptococcal infections, RF and RHD, was given by Dr. Bytchenko, Dr. Strasser and Dr.Czetnowitch of the W.H.O., Geneva, in August 1972. A two year pilot study was initiated in 23 villages near Vellore in June 1973 with support from W.H.O., Geneva. Support from the Indian Council of Medical Research (ICMR) from 1974-1975 enabled us to complete the pilot study. At the conclusion of the pilot study, the Ministry of Development Co-operation of the Government of Netherlands funded the originally planned surveillance study among the rural school children in a south Indian community for a period of four years.

Results of a four year surveillance study on streptococcal infections, RF and RHD, undertaken in children in the 5-15 year age-group is reported here. It has been found out that streptococcal infections and their sequelae are highly prevalent among rural children of southern India as will be presented in the subsequent chapters. An accurate assessment of the prevalence and incidence of RF and RHD has been established, supported by clinical, epidemiological and laboratory evidences. For the first time in India, it has been shown that RF and RHD can be prevented in a defined population, namely school going children, by timely primary prophylaxis and recurrences of valvular disease prevented by regular secondary prophylaxis.

Results of this multi-disciplinary study reveal that, inspite of social customs and ignorance, with a concerted attempt by epidemiologists and clinicians, well supported by laboratory investigations and the co-operation of parents, students and teachers, the rural population can be educated and will accept antimicrobial prophylaxis (primary and secondary). Hence, the incidence of initial attacks and recurrence of rheummatic fever can be prevented in India, as it has been prevented in developed countries of the world.

This study is the result of team work, intradepartmental and interdepartmental, where not only staff on the project but a number of other staff contributed much. We gratefully acknowledge the help of our colleagues in Microbiology, Community Health, Cardiology,

members of the departments of Biostatistics, Nephrology, Clinical Pathology, Clinical Biochemistry, Medical Records and many others who contributed to the various aspects of this study.

We would also like to place on record our gratefulness to Dr.L.B.M.Joseph, Director, Dr. A.S. Fenn, the then Principal, and the different Medical Superintendents of the Christian Medical College and Hospital, Vellore for giving us all help, encouragements and permission to use all the facilities in the institution to make this study a reality.

APRIL 1982 DR. GRACE KOSHI

Post Script

The manuscript of this report though ready in early 1982, unfortunately had to await till April 1987 for final printing, binding and release. This was necessitated because of repeated clerical staff changes, sabbatical leave of Chief investigator, retirements of co-investigators, as well as resignations of other senior and junior staff on the project. The final form of this report, though delayed, have been published in parts in National and International journals of repute; copies of available reprints are enclosed.

From the experience gained in this long term surveillance study, by our (study) team in Vellore from 1973-1978 and studies undertaken by the Indian Council of Medical Research at Delhi, has emerged two other ongoing community based Rheumatic fever control studies:-

(1) A multicentric collaborative study on "Development of a Demonstration Module for Feasibility of Primary prophylaxis, detection and Management of Rheumatic fever & Rheumatic Heart Disease in the community within the existing Health infrastructure". funded by the ICMR at Vellore, Delhi & Varanasi from 1982-87.

(2) A collaborative inter-departmental study funded by the Department of Science & Technology(DST) at Vellore from 1982 to Feb.1987 on "Rheumatic Fever, Rheumatic Heart Disease and streptococcal infections - Analysis of prevalance, evaluation of current methods of surveillance and research into alternative approaches in control".

The delay in this report was unavoidable and is very much regretted.

APRIL 1987

DR GRACE KOSHI

## ACKNOWLEDGEMENT

We gratefully acknowledge the impetus given by the W.H.O., Geneva, in initiating a Pilot Study from 1973 to 1975 on surveillance of streptococcal infection and sequelae among rural South Indian school children near Vellore. Our thanks are also due to the Indian Council of Medical Research, New Delhi who supported us from 1974-75.

Our grateful thanks overflow all bounds to the Ministry of Development Cooperation of the Government of Netherlands, for the timely and generous support which made the "Surveillance and Control of Streptococcal Infection and Sequelae" in the above community become a reality. We are also thankful to the Government of India, Ministry of Health and Family Planning (Department of Health) for permission to accept the Grant from Netherlands Ministry of Development Cooperation (vide No.U 11020/13/74-ME (U.G.) dated 25th March 1975).

Special thanks are due to the late Mr.S.G.Inbaraj and also to Dr.J.Richard and Prof.P.S.S.Sundar Rao of Biostatistics who helped with the statistical problems at every stage. The valuable help of Mr.C.C.Jacob and other members of the Accounts Department for their untiring help throughout the study is gratefully acknowledged.

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Special acknowledgement is made of the contributions and help given by many staff in Microbiology. The contributions of Dr. C.P. Thangavelu, Dr. K.N. Brahmadathan, Mrs. Rajeswari Pandian, Mrs. Sosamma John, and Mr. A. Kamalakaran have been invaluable throughout. Our thanks are also due to the help given by Dr. G. Sridharan at different stages. The valuable help rendered by Mrs. Kanthimathi & Swami Pillai is also acknowledged. The ungrudging help given by the departmental secretaries, Mr. P.M. Thangaraj during the earlier part of the study, Mr. A. Sivadas subsequently, in getting the annual reports & final report ready and to Mr. P.R. Narayana Murthy in the final part of the report, is gratefully acknowledged. The technical help of Mr. M. Kesavelu, Mr. D. Jesudason and other assistants of all grades were unmatchable & is gratefully acknowledged.

We are most thankful to all the school children, their parents and teachers for their cooperation throughout, without which this study would not have been possible. Last but not least our thanks are also due to all the Social workers, Field workers, & Drivers who made it easy for our team to carry on this community based project.

CONTENTS


| | | PAGE NO. |
|---|---|---|
| | PREFACE | |
| | ACKNOWLEDGEMENTS | |
| CHAPTER 1 | INTRODUCTION | 1 |
| CHAPTER 2 | SUBJECTS AND METHODS | 6 |
| CHAPTER 3 | COMPARISON OF THREE METHODS FOR TRANSPORTATION OF SPECIMENS | 26 |
| CHAPTER 4 | COMPARISON OF THREE METHODS FOR GROUPING OF STREPTOCOCCI | 39 |
| CHAPTER 5 | CARRIAGE OF BETA-HEMOLYTIC STREPTOCOCCI IN RURAL SCHOOL CHILDREN, IN VELLORE, SOUTH INDIA | 53 |
| CHAPTER 6 | STREPTOCOCCAL ANTIBODY IN HEALTH & VALUE OF MULTIPLE ANTIBODY TESTS | 62 |
| CHAPTER 7 | STREPTOCOCCAL PHARYNGITIS AND ITS CONTROL IN RURAL SCHOOL CHILDREN | 76 |
| CHAPTER 8 | STREPTOCOCCAL PYODERMA IN RURAL SCHOOL CHILDREN IN SOUTHERN INDIA & THE IMPACT OF PROPHYLAXIS | 99 |
| CHAPTER 9 | STREPTOCOCCAL PYODERMA IN NON-SCHOOL GOING AND PRE-SCHOOL GOING CHILDREN | 133 |
| CHAPTER 10 | RHEUMATIC FEVER AND RHEUMATIC HEART DISEASE IN RURAL SCHOOL CHILDREN IN KANIYAMBADI, SOUTH INDIA - PREVENTION & CONTROL, A SUCCESS STORY | 142 |

CHAPTER 11 EPIDEMIOLOGY OF STREPTOCOCCAL INFECTION AND SEQUELAE IN RURAL SOUTHERN INDIAN SCHOOL CHILDREN 168

CHAPTER 12 SURVEILLANCE OF STREPTOCOCCAL PYODERMA AND SEQUELAE IN A CLOSED COMMUNITY 187

CHAPTER 13 ATTEMPTS TO DETECT POST STREPTO-COCCAL GLOMERULONEPHRITIS (PSGN) 206

CHAPTER 14 SUMMARY, IMPACT & ACHIEVEMENTS OF STREPTOCOCCAL SURVEILLANCE PROGRAMME IN RURAL SOUTH INDIAN SCHOOL CHILDREN 210

CONCLUSIONS

APPENDICES

PAPERS PUBLISHED

# CHAPTER 1

## INTRODUCTION

Streptococcal infections are the most common of all bacterial infections of man and present a world-wide problem. strptococci are not only biologically diverse, but also diverse in the clinical manifestations they produce in man. Of all the streptococci, Group A streptococcus is the most important pathogen. Their importance is not only because of the variety of infections they produce at different sites of the body, but mainly because of the dreaded sequelae they produce.

Of the infections by group A streptococci, streptococcal pharyngitis is more comon in the temperate zones and streptococcal skin infections (pyoderma) rarer or present only in small pockets. However, the situation is different in the tropical and subtropical areas [1-5] where pyodermma is more common, and India is no exception. The dreaded sequelae of Rheumatic Fever (RF) and Rheumatic Heart Disease (RHD) are believed to follow streptococcal pharyngitis, whereas post-streptococcal glomerulonephritis (PSGN) may follow streptococcal pharyngitis or pyoderma.

The prevalence and incidence of RF and RHD are declining in most parts of the world from which data is available [7-9]. In Japan, the prevalence of RHD in school children has declined from 4.6 per 1000 in 1958 to 0.1 per

1000 by 1971. A 90% fall in the prevalence of RHD has been reported from Jerusalem between the 1950s and early 1970. In Nashville, U.S.A., the incidence of RF has decreased from the low 0.15 per 1000 in 1963 to 0.06 per 1000 in 1969. A similar declining trend has been observed in the U.S.S.R. A decline in the mortality and severity of the disease has also been noted in the U.S.A., England and Japan.

The information on streptococcal infections and sequelae, is rather limited, as far as India is concerned [10-13]. Data based on hospital population do not always reflect the actual epidemiological situation in the population [11,14-15]. The Indian subcontinent is a vast country with differfent climatic and socio-economic conditions and what is true for one area may not be the same for another area. There is no clear data on prevalence of RF and RHD undertaken by the Indian Council of Medical Research from 1974 to 1978 at Agra, Alleppey, Bombay, Delhi and Hyderabad revealed widely varying results. There were wide differences in the coverage of children from different categories of schools at different centres [16]. Moreover, not all the studies in the different centres were supported by proper laboratory data. Hence, the prevalence rates varied from as low as 1.8/1000 in Bombay to 11.1/1000 in Delhi and results could not be taken as conclusive.

1.1. Pilot study at vellore: 1973 - 1975

Realising the need for a well conducted epidemiological study on streptococcal infections, RF and RHD, supported by good laboratory data, a pilot study* was undertaken in 1973 in the Department of Microbiology, Christian Medical College & Hospital, Vellore, with the collaboration of the Departments of Community Health and Cardiology. Results of the pilot study using trained field workers[13] revealed the high prevalence of streptococcal pyoderma over streptococcal pharyngitis among rural children. The need for a more detailed surveillance study on streptococcal infections and sequelae among rural south Indian children was recognised in order to draw proper conclusions. The timely grant given by the Netherlands Government made such a study possible. A report on the streptococcal surveillance study undertaken from June 1975 through December 1978 is given herein.

1.2. Objectives

1.2.1. To establish the carrier-rate of beta-hemolytic streptococci (BHS) in the throat, nose and skin of normal, healthy rural school children.

---

* The Pilot study was initially supported by the World Health Organization, Geneva and in the second year by the Indian Council of Medical Research, New Delhi.

1.2.2. To establish the normal streptococcal antibody levels in healthy rural school children

a. Anti-streptolysin O (ASO)

b. Anti-hyaluronidase (AH)

c. Anti-deoxyribonuclease B (ADNB)

1.2.3. To detect all children with streptococcal pharyngitis and pyoderma, to establish their prevalence rate and to register such cases in the respective registry.

1.2.4. To detect and to establish the prevalence rate of RF and RHD in south Indian children, and to register and follow up all such cases.

1.2.5. To institute primary and secondary prophylaxis with Benzathine Penicillin and to study its impact on the further incidence of streptococcal infection and sequelae.

1.2.6. To carry out regular 3 to 4 monthly surveillance of all the normal and diseased children in order to establish:

a) Incidence rate of streptococcal pharyngitis

b) Incidence rate of streptococcal pyoderma

c) Incidence rate of RF and RHD

1.2.7. To study the epidemiology of streptococcal infections in south Indian children by:

a) documenting the serotypes of group A streptococci involved in pharyngitis and pyoderma as well as in RF and RHD

b) studying the host and environmental factors influencing streptococcal infection in a tropical population

1.3. Additional objectives

1.3.1. To evaluate and introduce newer and better techniques for:

a) transportation of clinical specimens

b) preservation of strains by sand desiccation and other techniques

c) preservation of strains and sera by lyophilization

1.3.2. To produce streptococcal antisera

1) Grouping sera

2) T-typing sera

3) Group B-typing sera

4) M-typing sera, if possible

1.3.3. To introduce newer tests reagents

a) Anti-DNase B reagents & test

b) Co-agglutination grouping with indigenous reagents

c) Streptolysin O antigen | production

d) Anti-streptolysin O standard |

# CHAPTER 2

## SUBJECTS AND METHODS

2.1. General Information

2.1.1. Area chosen and population

2.1.2. Home

2.1.3. Schools

2.2. Surveillance Methods

2.2.1. Metereological Data

2.2.2. Population Data

2.2.3. Protocols

2.2.4. Survey and Registration of subjects

2.2.5. Laboratory investigations

2.3. Bacteriological Procedures

2.3.1. Specimens for study

2.3.2. Collection of specimens

2.3.3. Transport of specimens

2.3.4. Media inoculation

2.3.5. BHS Recognition and recording

2.4. Serological Procedures

2.4.1. Specimens and Tests

2.4.2. Anti-streptolysin O titration

2.4.3. Anti-deoxyribonuclease B

2.4.4. Anti- hyaluronidase

2.4.5. C-reactive protein

## 1. General Information

### 1.1. Area chosen and population

The Christian Medical College Hospital, Vellore is in rth Arcot District in Tamil Nadu State and is 140 lometers south-west of Madras City (Fig.2.1). The mmunity Health Department of the Christian Medical College spital (CMCH), Vellore has been carrying out curative, omotive and preventive health services in a section of aniyambadi block, North Arcot District, Tamilnadu, India. he Kaniyambadi block, which is 15 kilometers south of CMCH, as a population of 80,000 and about 30,000 people are overed by the health services of the Government. The xisting health programme of the Community Health epartment (CHD) has helped the CHD to have a good rapport ith the community in the villages in Kaniyambadi block.

In consultation with the Departments of Community ealth and Biostatistics, it was decided to restrict the urveillance of streptococcal diseases to 13 villages in the aniyambadi block covered by the CHD. There are 15 schools n the 13 villages (Fig.2.2) and the children from these illages go to one or another of the schools in the articular locality. The main part of the study was carried ut among the children in these 15 schools.

2.1.2. Homes

The main entrance of a village, which is the centre of activity, is usually indicated by a temple (Fig. 2.3), and a central well where people gather for drawing water and the school is usually located nearby. About 65% of the houses in the study are mud-walled with grass roofs and mud flooring, plastered with cow dung (Fig.2.4). Nearly 70% of the houses have only one room (Fig.2.5) and a cattle shed is attached to one side of the house (Fig.2.6). A protected water supply is virtually non-existent and disposal of human and other wastes is very primitive. Flies and mosquitoes are abundant in the unhygienic surroundings.

Most of the people in these villages are landless labourers. Men earn about Rs.4/- to Rs.5/- per day and women Rs.1.50 per day. Agricultural work is seasonal and the women are unemployed for at least 200 days of the year. Men are fairly regularly employed during the agricultural seasons.

The infant mortality rate is still as high as 120 per 1000 and the birth rate about 39 per 1000. The major causes of death are diarrhoea, malnutrition and respiratory infections. There is a high incidence of viral infections including complications, tuberculosis, skin and throat infections, nephritis and heart disease. Poverty, ignorance and wrong beliefs are the major reasons for the state of poor health of the people.

2.1.3. Schools

Generally there is only one school in a village, usually a primary (elementary) school. A larger village has a secondary school and an occasional one a high school. The schools are usually pakka with tiled roofs and plastered walls: Occasionally a school will be mud walled and mud floored and/or thatched (Fig.2.7). Classroom divisions are often with bamboo screens (Fig.2.8) or with half walls. At times, 2 or 3 divisions of children are accommodated in a single room and children of lower classes squat in the class rooms with no provision for benches or chairs and tables (Fig.2.9). Certain classes are held in the open air under trees and different classes take their turn for this as needed (Fig.2.10).

There is no provision for a protected water supply in the schools. Very rarely there are school wells, but often water is kept in mud pots. Insects, especially flies and mosquitoes, are abundant and the surroundings are unhygienic.

Though there is provision for the medical inspection of school children as they enter and leave the school, in practice this does not seem to be carried out, because there are only 2 doctors for the population of 80,000 and its 15 schools in the Panchayat Union. Further more, it takes time to gain the understanding and co-operation of the teachers and the parents.

Infants are usually looked after by the elder siblings when the parents go for work. This prevents the older children, particularly the girls, from attending schools regularly. Many join the school in the beginning and then attendance becomes seasonal according to the seasonal work of the parents.

## 2.2. Surveillance methods

### 2.2.1. Metereological data

In the villages chosen for the study, the daily temperature varied from 18-20 $^{o}$C in winter, and 26-40 $^{o}$C in summer. The average rainfall per year was 431 mm. The area suffers from periods of drought and famine.

### 2.2.2. Population data

The population data was updated. The detailed streptococcal surveillance programme was limited to children upto 15 years of age. To get the maximum coverage of children belonging to each village, we planned to carry out the survey as follows:-

| | |
|---|---|
| School-going children | - to survey all in the 15 rural schools |
| Non-school going children | - to survey the pre-school (0-5 Yrs) and 5-15 Yrs old, non-school going children in their homes in representative villages |

As most villagers are farm workers, they leave home by 6 A.M. or earlier. Contacting them during normal working hours between 8 A.M. and 4 P.M. was found to be not feasible. Therefore, for part of the initial survey, the streptococcal team undertook to work in the villages from 6:30 A.M. and were able to do the maximum coverage of children at homes.

2.2.3. Protocols

Five different types of protocols were designed for use in the programme, copies of which are found in the Appendix I a, b, c, d and e.

a. Family Card - Master form for the family

b. Member Card - For each child seen at school or home 0-15 Yrs.

c. Pharyngitis Card | - PP

d. Pyoderma Card |

e. Rheumatic fever and Rheumatic heart disease Card - RF

f. Acute glomerulo-nephritis - AGN

2.2.4. Survey and Registration of Subjects

The field workers, visited each home and school for complete data collection. The first few months were taken for preliminary data collection. The Family Card was filled in for details of all members and of their environment. At the school, or when non-school going children were seen at home, the Member Card was filled in for all details. Population data is given in Table 2.1 and 2.2.

Whether at the school or at home, every child was seen within a day or two by the Medical Officer, the history verified, a clinical examination done and the subjects categorized and registered as follows:-

| | |
|---|---|
| Normal controls | - Children with no obvious signs and symptoms of streptococcal pharyngitis, skin infection or sequelae |
| Streptococcal infection | - Pharyngitis or pyoderma |
| Streptococcal sequelae | - Rheumatic fever (RF)<br>Rheumatic heart disease (RHD)<br>Acute glomerulonephritis (AGN) |

2.2.5. Laboratory investigations

All necessary laboratory investigations were carried out. Swabs for culture and blood for serology were collected from cooperating children categorized as children with pharyngitis and pyoderma as well as those suspected to have sequelae of streptococcal infection (Refer Section 2.3 and 2.4 for details).

In addition, for those suspected of RF, RHD and PSGN , all additional investigations necessary were carried out as detailed in Chapter 9.

## 2.3. Bacteriological procedures

### 2.3.1. Specimens for study

Because of the difficulty in acceptance by the villagers and school children, it was decided to collect clinical specimens - swabs for culture and blood for antibody studies - from different categories of subjects as follows:-

| | |
|---|---|
| Normal control | - throat swabs from all |
| | - nose and skin swabs; and blood when possible from every fifth normmal healthy child |
| Pharyngitis | - swabs from throat and nose; blood for antibody studies when possible |
| Pyoderma | - swab from skin, throat and nose; blood when possible. |

### 2.3.2. Collection of specimens

Nontoxic (sulfur di-oxide free) cotton wool swabs, sterilised by autoclaving, were used throughout. Great care was taken with the method of collection and transport of specimens.

For collection of throat swabs, the mouth was well opened with adequate illumination of the pharynx. Both the tonsils and the posterior pharynx were rubbed well. In the case of pharyngitis, care was taken to touch the follicles as well as to collect the exudate if present. Care was taken to avoid touching the uvula, cheeks and the tongue.

For nasal swab collection, the nose tip was raised by one hand, the swab, moistened in sterile water or saline and then squeezed out, was gently introduced along the floor of one nasal cavity and then into the order, and gently swabbed to collect the specimen.

For the collection of swabs from normal skin in controls, swabs were moistened with sterile normal saline, the excess squeezed out, and multiple 4x4 cm square sites, including ankles, wrists, back of the ears and neck, were swabbed.

From pyodermma lesions, if at the vesicular stage, the outside of vesicles were gently cleaned with sterile saline, the vesicle punctured and squeezed gently and the pus collected on the swabs. In crusted lesions, the crust was carefully removed and floor of the crust was gently and firmly rubbed with the swab to collect the pus. If the lesion was dry, a moistened swab was used to collect the specimen.

2.3.3. Transport of specimens

Streptococci do not survive well on wet cotton or dacron swabs. Hence, if a delay of more than 2 hours is expected to elapse between specimen collection and inoculation into media, proper methods of transportation such as transport in Stuart's media or other methods must be employed. We compared the filter paper (FP) technique of transport of Hollinger et al (Refer Appendix III) with inoculation of swabs on blood agar (BA) in the field (IF), and inoculation on BA in the laboratory (IL) within 4 to 5 hours of collection on the dry cotton swabs. As the streptococci survive well in a dry environment and since the filter paper technique was found superior to the IF and IL methods, the FP technique was followed for the subsequent study (Refer Chapter 3).

2.3.4. Media Inoculation

Streptococci grow well on blood agar. Blood agar with 5 to 7% sheep blood is the best. Horse blood may be used equally well. Human blood is inhibitory and should not be used[2]. We used sheep blood agar, pH 7.3 to 7.4 throughout the study (Appendix II). The plates were incubated at $35^{o}C \pm 1^{o}C$ aerobically or occasionally in $CO_2$ atmosphere, for overnight. If negative for beta-hemolytic streptococci[2] (BHS)[3,4], the plates were further incubated for 48 hours

For the cultivation of specimens from nose and normal skin, selective media were used. For this, crystal violet blood agar (CVBA) plates (Appendix II) were used, as the crystal violet suppressed the growth of staphylococcal contaminants from the skin, which may overgrow and mask the streptococci.

When BA or CVBA was inoculated the primary streak was cut in with the loop once or twice, as the BHS produce better hemolysis in the depth of the medium. The CVBA were incubated as for BA cultures.

For details on the FP technique of culturing refer to Chapter 3 and Appendix III.

2.3.5. BHS recognition and recording

BHS grow on BA within 18 to 24 hours and rarely after 48 hours as typical translucent, small, convex colonies surrounded by clear beta-hemolysis. The small (1 to 2 mm), smooth, "glossy" colonies were more often encountered. Rarely were larger smooth "mucoid" flowing colonies with beta-hemmolysis as well as "post-mucoid" or dry "matt" colonies encountered. Only BHS were further followed up for characterization. Rarely were alpha hemolytic or non-hemolytic colonies further characterized.

Quantitative estimation of growth obtained were recorded as follows:-

| | |
|---|---|
| 1 to 20 colonies | + |
| 21 to 100 colonies | ++ |
| > 100 colonies | +++ |

Quantitation of growth in the FP method of culture was not accurate. Wherever required, colonies were subcultured for purity. The purified colonies were further followed up for antigenic grouping and typing. (Refer Chapter 4 and Appendix III). Antisera for group identification as well as indentification of T-antigens of group A streptococci were locally produced and standardised (Refer Appendix V).

The BHS isolates were preserved by using different techniques (Refer Appendix VI).

## 2.4. Serological procedures

Streptococcal antibody determination is of value in confirming streptococcal infection or their sequelae [5,6]. The antibody response against the various streptococcal antigens depend on the site of infection, the nature of the sequelae which follow and also by the type of test used to detect the antibody. The value of multiple streptococcal antibody tests, particularly in pyoderma and in the sequelae of streptococcal infections, has been pointed out by many workers [7-10]

2.4.1. Specimens and tests

Whenever possible for this study about 5 ml of blood was collected by venipuncture from children with pharyngitis, pyoderma or suspected RF, RHD or AGN. Although attempts were made to obtain blood from normal healthy children to be included as 'Controls', very few healthy children or their parents permitted blood collection.

All blood specimens were transported to the laboratory within a few hours of collection. Serum was separated soon after receipt in the laboratory, and preserved at 4 °C. Whenever testing was not possible in a few days, serum samples were preserved at -20 °C until the time of testing.

Whenever possible, tests were carried out for anti-streptolysin O (ASO) anti-deoxyribonuclease B (ADNB) and anti-hyaluronidase (AH). Sera from children suspected to suffer from sequelae such as RF, RHD or AGN were also tested for C-reactive protein (C-RP).

2.4.2. Anti-streptolysin O (ASO) titration

ASO test was carried out by the microtiter technique[11]. The streptolysin O (SLO) antigen for use in this test was prepared and standardised in the laboratory and was preserved at 4 °C, used for a period of 1 month and also as lyophilized SLO. Sodium dithionate ($Na_2S_2O_4$) was added to the SLO in a final concentration of 0.07% just prior to use (See Appendix IV for details). Microdiluters, dropper pipettes and other accessories for the microtiter technique

were from Cooke Engineering Co., Alexandria, VA, U.S.A. For details on the micro-ASO test refer Koshi[11] and Appendix V.

2.4.3. Anti-deoxyribonuclease B (ADNB)

The ADNB test was carried out by the microtiter method similar to that described by Nelson et al.[8]. The DNase B enzyme employed as antigen in this test was produced in the laboratory from group A streptococcus strain C203S. The enzyme precipitated from culture filtrate was purified by column chromotography and standardised against an ADNB standard supplied by Dr. J. Rotta, Chief, WHO Collaborating Centre for Reference and Research on streptococci, Prague. Details of reagents used, purification of DNase B and its standardisation, as well as ADNB titration of serum samples, are given in Appendix V.

2.4.4. Anti-hyaluronidase (AH)

The AH test based on mucin-clot prevention technique was also carried out by microtiter method as described by Murphy[12] using commmercially (Difco, USA) available reagents. An account of the methodology employed is given in Appendix V.

2.4.5. C-reactive protein (C-RP)

Test for C-RP was carried out by capillary precipitation using commercially available reagents (Difco Laboratories). See Appendix V for details.

TABLE 2.1
POPULATION DATA-SURVEILLANCE STUDY

| | NO. | PERCENT |
|---|---|---|
| VILLAGE | 13 | |
| FAMILIES | 2499 | |
| MEMBERS | 12670 | |
| CHILDREN 0-15 Yrs | 5331 | 42.1 |
| MALE | 2746 | 51.5 |
| FEMALE | 2585 | 48.5 |

TABLE 2.2

DATA ON CHILDREN INCLUDED IN THE STUDY

| | NUMBER | PERCENT |
|---|---|---|
| SCHOOLS SURVEYED | 15 | |
| CHILDREN SURVEYED AT ANY TIME | 3890 | |
| MALE | 2283 | 59 |
| FEMALE | 1607 | 41 |
| CHILDREN SEEN IN 1st SURVEY | 2577 | |
| MALE | 1541 | 60 |
| FEMALE | 1036 | 40 |
| CHILDREN SURVEYED AT HOME | 2015 | |
| PRESCHOOL CHILDREN (0 - 5 YRS) | 1281 | 63.6 |
| NON-SCHOOLGOERS (5 - 15 YRS) | 734 | 36.4 |

FIG. 2.1

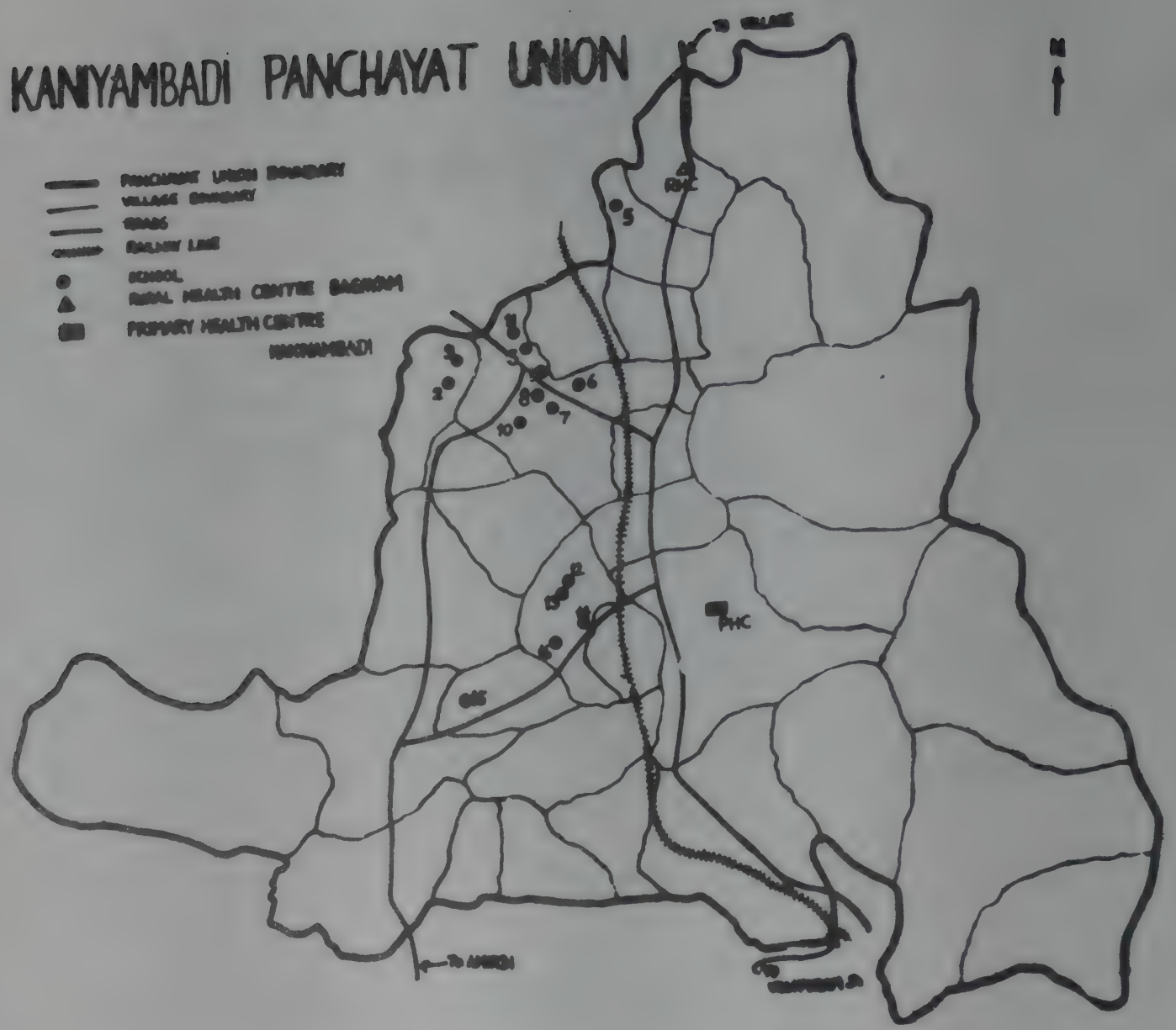


FIG. 2.2.

MAP OF KANIYAMBADI BLOCK SHOWING THE 15 SCHOOLS SURVEYED

FIG. 2.3.

TEMPLE AT THE ENTRANCE TO A VILLAGE

FIG. 2.4.
A TYPICAL VILLAGE STREET

FIG. 2.5.
ONE-ROOMED HOUSE OF A
RHD PATIENT

FIG. 2.6.
A VILLAGE HOME WITH
ATTACHED CATTLE-SHED

FIG. 2.7.
A VILLAGE SCHOOL WITH THATCHED ROOF

FIG. 2.8.
A CLASS ROOM WITH BAMBOO
SCREEN SEPARATION

FIG. 2.9.
TWO CROWDED CLASSES BEING
HELD IN THE SAME ROOM

FIG. 2.10.
AN OPEN AIR CLASS IN SESSION

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# CHAPTER 3

## COMPARISION OF THREE METHODS FOR TRANSPORTATION OF SPECIMENS FOR CULTURE OF BETA-HEMOLYTIC STREPTOCOCCI

Results of culture for streptococci will depend on the best way of collection of swabs, especially throat swabs from children, transported without much delay in an appropriate manner and culture on blood agar containing 5 to 7% sheep blood or horse blood. Different methods of collection and transport of throat swabs have been evaluated by various workers [1-3]. The filter paper (FP) strip technique of Hollinger and associates [4-5] has been recommended as a simple and cheap technique for the transport of streptococci. The value of silica gel dry-swab transport [6,7,8] and Stuart's transport media [4] have also been studied by various workers. Results obtained by others may not be wholly applicable to tropical condition, where variations in temperature and humidity affect the viability of various microorganisms.

At the beginning of the streptococcal surveillance programme, we undertook an evaluation of various techniques of the transportation of swabs with other methods so as to use the better technique for this long term study. The usefulness of the economical and easier method of filter paper(FP) transport against two other techniques namely, inoculation in the field(IF) on sheep blood agar (BA)

immediately after collection of the swab and transport of the swab to the laboratory and subsequent inoculation in the laboratory, were evaluated[9]. Results of the study are presented here.

## MATERIALS AND METHODS

The study was carried out in two phases. In the first phase of the streptococcal surveillance programme, different methods of transportationn of throat and skin swabs were done in November and December 1975 when the temperature varied between 28-31 °C. Throat swabs were collected using previously tested non-toxic cotton swabs under direct vision, swabbing both tonsillar and post-tonsillar fossae and posterior pharynx without touching the tongue or cheeks. From the skin lesions, material was taken with cotton swabs, taking care to get the serous fluid or the pus from underneath the crusts. Three swabs were held together when specimmens were collected from throat or skin lesions. One swab was directly inoculated on a 5% sheep blood agar (BA) immediately after collection in the field (IF). Material from the second swab was transferred to a filter paper strip (FP) and the third swab kept in a sterile test tube, and were then transported to the laboratory (IL). Both the FP and IL were cultured on BA within 4 to 5 hours. All BA plates were incubated aerobically at 35 °C ± 1 °C for 18 to 24 hrs. The growth of beta-hemolytic-streptococci

(BHS) were graded as scanty (1 to 20 colonies), moderate (21 to 100 colonies) or heavy(>100 colonies), if on a regularly streaked plate. On BA with FP technique it is difficult to assess growth quantitatively. Cultures negative for streptococci were incubated for a further 24 hrs.

In the second phase of the study undertaken in March and April 1976 (35-38 C), three swabs were collected from lesions of children one after another, to eliminate errors connected with simultaneous collection of three swabs. Each swab was coded as 1,2,3 and subsequent processing done as IF, FP, IL; FP, IL, IF OR IL, IF, FP in that order. Incubation and reading of results were carried out as in phase 1. In the usual FP technique, the FP strip smeared with the clinical material, on reaching the laboratory, is gently placed on one half of a BA plate, the pencil marked side facing up. After 4 hours' incubation the FP is transferred to the fresh half of the BA.

## RESULTS

The results of the different methods of transport of swabs in the streptococcal surveillance study are given in Table 3.1 and 3.2. In phase 1 of the study, swabs of skin lesions from 55 patients were taken and in phase 2 swabs from 106 patients were used, so that a total of 161 specimens were tested by all 3 methods. Recovery of BHS from skin lesions in phase 1 was 82% and in phase 2, 80%.

Results show that 100 per cent recovery of BHS from skin lesions was obtained when swabs were inoculated directly in the field, in both phases of the study. When material from skin lesions was transported by FP or IL techniques, the yield was 97.8% by both in phase 1 and 95.3% and 96.5% respectively in phase 2. The yield of streptococci from skin swabs by the different methods in phase 1 and phase 2 or between the results of phase 1 and 2 were not statistically significant (Table 3.1). On the other hand, out of the 122 throat swabs tested, 76 in phase 1 and 46 in phase 2, the recovery of BHS was maximum by the FP technique followed by IF, and least by IL technique in both phases(Table 3.2). In the commonly iused FP technique the FP is removed and discarded into a lotion jar with a forceps; after overnight incubation one can see confluent growth of beta-hemolytic colonies in both the areas, second area showing heavier and pure growth (Fl. 3.1).

We have tried using smaller FP strips on half BA plates. The time of primary incubation with FP was reduced to 2 or 3 hours and after the FP is removed inoculation from the area for isolation of colonies was tried out. We found that incubation with the FP strip for 2 hours was not as good as after 3 or 4 hours. Moreover, the growth and isolation when FP is removed after 2 hours' incubation on half plate was found to be adequate and yielded isolated colonies on half plate itself.(Fig. 3.2.).

## DISCUSSION

Silica gel transport method was found good by Taplin and Landsdell[7] for transporting skin and throat swabs for streptococcal study. They founnd that while the direct plating yielded 78% recovery rate, the yield was 76% when delayed culture of the silica gel dry swabs were done. However, there was a slight reduction in the number of colonies recovered from silica gel transport technique. On the other hand, Hollinger and Linderberg[4] found the filter paper strip method of transport superior to the immediate plating and silica gel dry-swab technique. Jackson[8] found the filter paper strip technique as useful as silica gel transport in a study at Colorado.

In the evaluation of three methods of transport of skin specimens in phase 1 of our field study, there was no statistically significant difference between the percentage recovery of streptococci by the different methods of transport for skin swabs. Eighty to 82% of the skin cultures, yielded streptococci with no contamination by skin flora, even in hot and humid conditions. Subsequently we have found that the use of crystal violet blood agar minimised the problem of contamination with normal skin flora.

In contrast, in the case of well-taken throat swabs, the maximum yield was obtained by FP in both seasons, which was much higher than IF or IL. The transport of swabs to

the laboratory and subsequent inoculation within 4-5 hrs yielded very poor results in the case of throat swabs-59% in the fairly cold season and as low as 45% in the early hot and humid season. Results obtained by FP technique in phase 1 was significantly higher than IF ($P<0.05$) or IL($P<0.01$) techniques by the McNamara's test of significance for related observationns. In phase 2 also, results by the FP and IF were significantly higher than IL($P<0.01$; $P<0.05$ respectively). However, results were not significantly different between phases 1 and 2 of the troat swab study, just as with skin swabs. In throat lesions, where streptococci may be present along with normal flora, it is obligatory that swabs be collected under proper vision and transported to the laboratory quickly by the best possible method. Our studies also have confirmed the futility of dry throat swab collection and delayed inoculation in the laboratory. The study also revealed the problems of carrying large loads of blood agar plates and the possibility of contamination in a tropical, dusty atmosphere in the field. Further, since material is dried on the filter paper during the process of inoculation by swabs, the viability of streptococci is not affected within a few hours or even for a few days, whereas it inhibits mouth flora by drying [10].

The adequacy and superiority of filter paper strip method of transportation of skin and throat swabs under varying climatic conditions was thus confirmed in our study.

Whether swabbing of lesions was done with 3 swabs simultaneously (phase 1) or one after another (phase 2), it did not affect the yield of streptococci from skin or throat lesions. However, the one disadvantage of filter paper strip method is that the precise colony count cannot be done. In the case of throat cultures, colony count cannot be done. In the case of throat cultures, colony count is important to differeentiate carrier state from active infection. However, evaluation of growth as scanty, moderate or heavy was not difficult. Based on the results obtained, the FP strip method of transportation was followed in our continuing streptococcal surveillance study.

It was interesting to find in a later study that the FP technique could be utilized for obtaining isolated colonies from primary area by removing the FP after 3-4 hours' incubation, streaking from that area for isolation and incubating further overnight. Media can be saved by using half BA plate for one FP strip and subsequent inoculation.

An experiment on the effect of humidity on survival of different groups of streptococci revealed that streptococci, regardless of their group, could remain viable for a period of 21 to 27 days under low humidity at 25-31 $^{o}$C. Under high humidity, groups A and C survived only 10 days. But groups B and G survived for 14 days under high humidity at 25-31 $^{o}$C (Table 3.3).

Thus our study on comparison of techniques for transportation of clinical specimens has shown that the FP technique is the easiest, economical and most reliable technique, even under high humidity. Moreover, the modification of the FP technique showed that the original half of BA plate could be used better by removal of FP after 2 hours' primary incubation and then inoculating to get isolated colonies by overnight incubation.

TABLE 3.1

COMPARISION OF METHODS FOR THE RECOVERY OF BHS FROM SKIN SWABS

| STUDY | NO. DONE | PERCENT ISOLATION | | |
|---|---|---|---|---|
| | | FP | IF | IL |
| PHASE I (28-31 °C) | 55 | 97.8 | 100 | 97.8 |
| PHASE II (35-38 °C) | 106 | 95.3 | 100 | 96.3 |

FP= Filter Paper technique
IF= Inoculation in the field
IL= Inoculation the laboratory

TABLE 3.2

COMPARISION OF METHODS FOR THE RECOVERY OF BHS FROM THROAT SWABS

| STUDY | NO. DONE | PERCENT ISOLATION | | |
|---|---|---|---|---|
| | | FP | IF | IL |
| PHASE I (28-31 °C) | 76 | 97.1 | 76.5 | 58.8 |
| PHASE II (35-38 °C) | 46 | 89.7 | 72.4 | 44.8 |

TABLE 3.3

SURVIVAL OF DIFFERENT ANTIGENIC GROUPS OF STREPTOCOCCI ON FILTER PAPER STRIPS KEPT UNDER VARYING HUMIDITY AT ROOM TEMPERATURE

| GROUPS | NO. OF DAYS SURVIVED | |
|---|---|---|
| | LOW HUMIDITY | HIGH HUMIDITY |
| A | 27 | 10 |
| B | 25 | 14 |
| C | 27 | 10 |
| O | 21 | 14 |

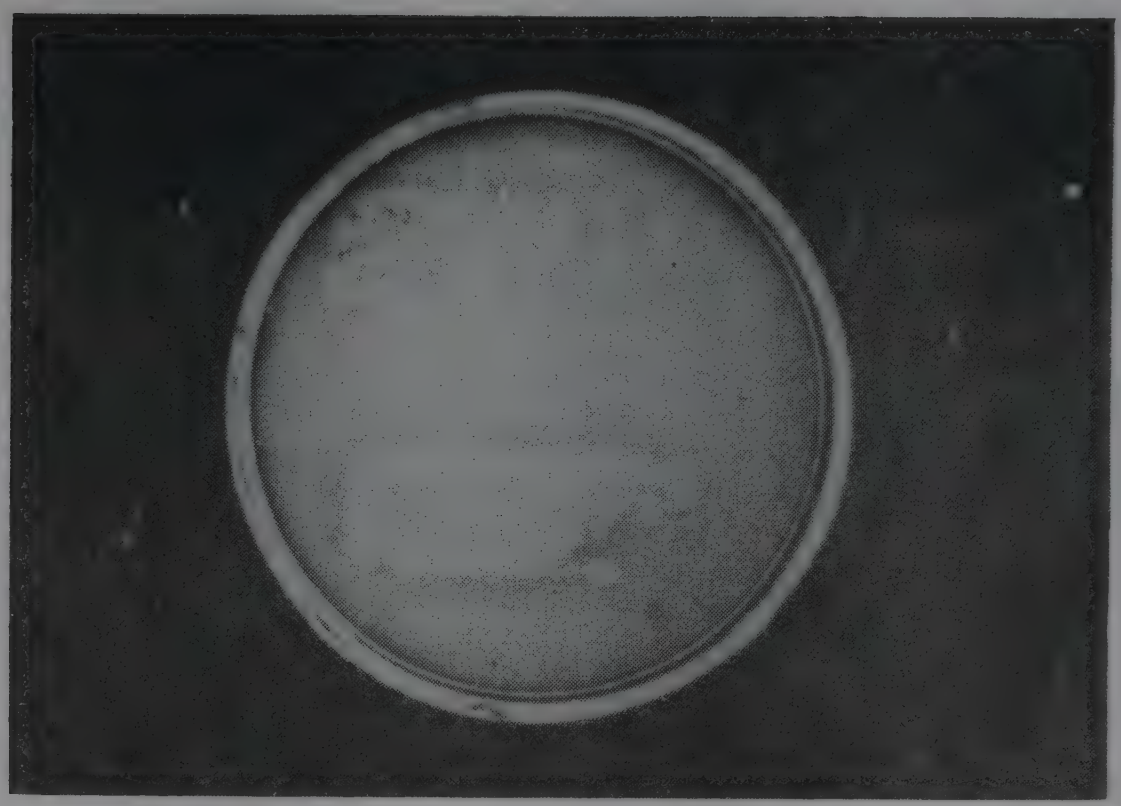

FIG. 3.1.

COMMONLY USED FILTER-PAPER CULTURE METHOD.
UPPER HALF: FP REMOVED AFTER 4 HOURS INCUBATION
LOWER HALF: AFTER ONE NIGHT INCUBATION

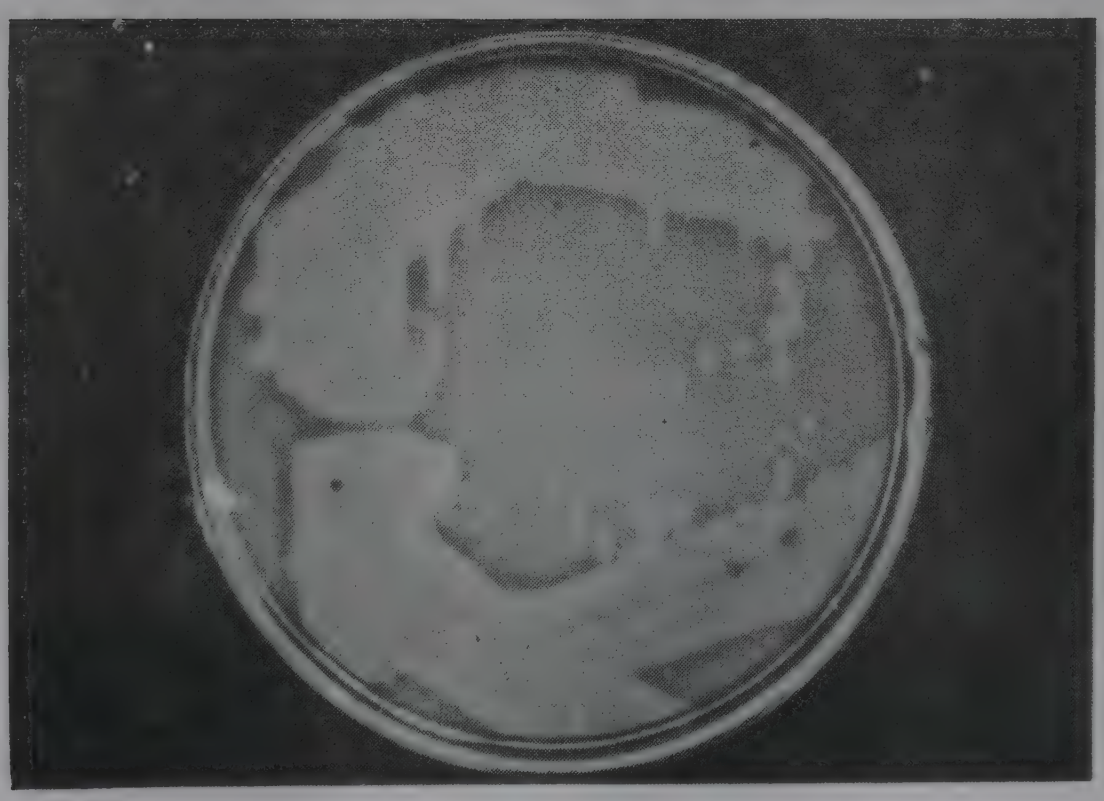

FIG. 3.2.

MODIFIED FILTER PAPER CULTURE METHOD
FP REMOVED AFTER 2 HOURS AND STREAKED FOR
ISOLATION OF HALF OF THE PLATE

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# CHAPTER 4

## COMPARISON OF THREE METHODS FOR GROUPING OF STERPTOCOCCI

Group A beta-hemolytic sterptococci (ABHS) or Group A streptococci (GAS) are among the most important agents of human infections. They produce not only acute tonsillo-pharyngitis and skin infections but also rheumatic fever (RF) rheumatic heart disease (RHD) and post-streptococcal glomerulonephritis(PSGN). In recent years, many reports have appeared regarding the importance of group B streptococci (GBS) in neonatal menigitis and septicemia[1,2,3,4]. Group G was involved in an epidemic of pharyngitis[5] and infections of the skin and other sites by group g are known[6]. Group C and F are also involved in various infections and the latter more often producing deep seated purulent infections[7,8]. With the importance of not only ABHS but also other groups of streptococci in various human infecions, it is important that clinical laboratories identify all common groups of beta-hemolytic streptococci. The universal method of grouping streptococci, is the time - old precipitation test using hot acid extract in capillary tubes[9]. Grouping by precipitation is done by a ring test with hot acid, formamide and other extracts. Agar gel precipitation[11,12] and coagglutination technique has also been tried[13].

During part of the streptococcal surveillance, we carried out comparisons of gel-precipitation and coagglutination technique with capillary precipitation and a report is made of the findings.

## MATERIALS AND METHODS

For the capillary precipitation test, hot hydrochloric acid extracts were prepared from 30 ml Todd Hewitt Broth (THB) culture sediment. Specific grouping sera of good potency were prepared in this laboratory from groups A, B, C, F and G (Appendix V). The capillary tubes with the serum extract mixture were inserted onto a roll of plasticine in a wooden board so that serum was above the extract. Reactions were read within 1 to 5 minutes.

For grouping by precipitation in agar-gel, a Gelman immunodiffusion kit was used (refer Appendix III). Standard procedues of immuno-diffusion were followed [12]. The gel was prepared from Difco Nobel agar at 1% concentration in 0.85% saline solution and 0.01% sodium azide as preservative. Results were read after overnight incubation at 4°C.

For the coagglutination technique of grouping, Protein A containing Staphylococcus aureus Cowan I strain was grown overnight in THB with 1.5% agar (refer Appendix III). For sensitization 0.1 ml of the streptococcal grouping serum was added to 1 ml of 10% staphylococcal suspension and left at room temparature for 30 minutes. The sensitized staphylococci were washed and resuspended to 1% in PBS containing 0.1% sodium azide. This suspension was found stable for many months at 4°C.

Grouping by coagglutination was done by placing a drop of the staphylococcal reagent in each ring of a ceramic ring slide. A drop of the culture of streptococci, growing in 2 ml THB at 35°C ± 1°C overnight, was mixed with the reagents for each of the commonest groups in each ring. The slide was rotated normally for 1 minute and the results were read over a slide viewer. The agglutinating clumps were easily seen and the degree of macro agglutination was read as 4+, 3+, 2+, 1+ and ± based on the size of the clumps and clearing of the mixture. Negative reactions showed a uniform suspension and was read again after 2 minutes' rotation. Cross reactions were overcome by using absorbed and diluted grouping sera for sensitization for groups B, C, D and G. Trypsinization of the culture for 30-60 minutes overcame the problem of cross reaction in some autoagglutinating strains of groups A and G. Group F never gave cross reactions.

## RESULTS

A total of 170 strains, mainly from throat and skin lesions, were tested by the above 3 methods. The capillary precipitation test with hot acid extract gave clear-cut quick results within 1 to 5 minutes (Fig. 4.1). There were no cross reactions. Rarely was further incubation upto 2 minutes needed. Grouping by precipitation in agar gel using the same acid extract also gave very clear-cut precipitin lines after overnight incubation at room temperature

(Fig.4.2). Group B gave diffuse and fainter reactions, but were never missed.

Results of comparision of grouping by precipitation in agar gel with the conventional capillary tube precipitation and grouping by coagglutination technique is given in Table 4.1. There was 100% agreement of agar gel and 99.4% agreement of grouping by coagglutination with capillary precipitation.

Reactions by coagglutination technique of grouping was easily recognizable. All but one strain of group C out of the 170 tested were correctly grouped by coagglutination. Most group F strains needed 48 hours incubation in THB to get enough cell concentration for tests. Group F needed 2 min. of mixing for grouping by coaggulation method. There was minimal cross reactions with some strains. However, the homologous reactions were most oftern 3+ to 4+ and easily and quickly recognised (Fig.4.3).

The cross reactions with heterologous strains were slow in developing and rarely the reactions exceeded $\pm$ to 1+ except in one group C strain which gave 3+ reaction with all. The cross reactions seen with this strain disappeared when the strain was subcultured and tested again. Group B and F never showed any cross reactions. The cross reactions of group A shown with group C reagent were removed by preabsorption of the Group C serum with Group A cells. In others, use of diluted antiserum for coating the staphyloco-

ccal reagent was adequate to overcome the cross reactions. The heterologous reactions shown by group G strains which were of an autoagglutinable nature were removed by trypsinization of the test strains. A preliminary trial with 75 strains showed that grouping by coagglutination could be done in 73 strains with 4 to 6 hour old THB culture suspension. A subsequent extension of the study revealed that 62% of 320 strains tested could be grouped by coagglutination in 4 hours, 82% in 6 hours and all by overnight incubation. In the case of group B where growth was rapid 93% could be grouped in 4 hours and 97% in 6 hours. Influence of the period of incubation on grouping of streptococci by coagglunation is given Fig. 4.4.

## DISCUSSION

Lancefield's method of extraction of carbohydrate antigens by hot hydrocholoric acid and its detection by capillary percipitation is the most universally employed technique of grouping of streotoccoci[9]. For reliable results, the grouping sera have to be highly potent and are to be used undiluted. Problems with some commercial grouping sera have been highlighted[14]. The performance of the extract advocated by Elkholy and co-workers in the capillary precipitation was also found to vary with the potency of the grouping sera used[15]. Moreover, though the Elkholy method of nitrous acid extraction is simple and quick, the amount of carbohydrate (extracted was lower than the Lancefield extract, and some commercial sera may

not be potent enough for its detection[14]. The capillary precipitation technique, however needs abundant bacterial growth in 30 ml of THB and overnight incubation, for the extraction of the group specific carbohydrate.

The grouping sera was used were produced locally and were found to be highly potent and could be used unabsorbed and did not give any cross reactions by the capillary or gel precipitation technique. The agar gel precipitin lines were well-defined and clear-cut. The saving of grouping sera is advantageous, but the results can be read only after overnight incubation. Moreover, considerable time is needed in the precipitation of the gel and in setting up of the test. The contention that unabsorbed and even cloudy sera can be used in agar gel and no cross reactions occurs has been put forward in favour of agar gel precipitation for grouping[11,16]. The potent sera we raised locally could be used unabsorbed for capillary precipitation, with no heterologous reactions and results were ready within 1 to 5 minutes of putting up of the test. Hence we find that there are definite advantages for capillary precipitation over agar gel precipitation for grouping. However, the advantages of agar gel precipitation for M-typing of ABHLS using unabosrbed sera are undoubted and unique as advocated by Rotta and co-workers[17].

The advantages of grouping by coagglutination are many. Only minute amounts of grouping sera are needed to sensitize the staphylococcal reagent and the sera can be

used diluted several times. The amount of THB needed for growing the streptococci for coagglutination grouping is 1 ml as against 30 ml of THB for Lancefield extraction and precipitation grouping. Further, the coagglutination grouping is rapid and the results are avilable in 4 hours in 62% of strains. The method is highly reliable and we found that 100 per cent of this large series could be correctly grouped after overnight growth in THB[18]. It was highly significant that even the two strains of group D which could not be grouped by hot acid extraction and capillary precipitation could be grouped by coagglutination from 1 ml THB culture. Besides, the coagglutination grouping of streptococci is simple to perform, does not need sophisticated equipment or procedures and can be done on an ordinary microscope slide, or on a ceramic ring slide or on a slide with concavity[19,20]. We also found that multiple cultures could be easily tested on one concavity slide or ring slide as in the case of the VDRL test. The macroagglutination results are usually strong, easily visible and interpretted[19,20].

Most other reports are based on the use of commercially available reagents and highlight the presence of cross agglutination shown by certain strains, which could be easily differentiated from the strong agglutination seen with the homologous group reagents. The cross reactions we encountered were only ± or + reactions except in the case of one Group C strains. The cross reaction of

the single Group G strain disappeared when subcultured and could not be reproduced. We could remove the weak cross reaction shown by some of our Group A strains by absorption of the Group C antiserum with Group A cells prior to the coating of the staphlococcal cells. Cross reactions shown by GRoup G strains were found to be of an autoagglutinable nature and were removed by trypsinization of the cells. Thus none of the cross reactions was a major problem in our hands using the sera we raised and all strains tested were correctly grouped by coagglutination.

The fewer number of cross reaction (5.2%) seen on the coagglutination tests in this study is due to the use of absorbed and/or diluted antisera for sensitization of the staphylococcal reagent. However, we found that homologous reactions were strong and heterologous reactions minimal when a properly standaradized staphylococcal reagent was used. In our hands, none of the group D and F strains showed any cross reactions seen with some strains were obviated by trypsinization of the streptococcal cells prior to coagglutination testing. Thus we have found that the coagglutination method of grouping of streptococci is highly reliable, specific and rapid and we recommed that coagglutination method of grouping streptococci for any routine laboratory.

TABLE 4.1

COMPARISON OF CAPILLARY AND GEL PRECIPITATION WITH COAGGLUTINATION WITH GROUPING

| GROUPS OF STREPTOCOCCI | NO. TESTED | PERCENT GROUPED: PRECIPITATION — CAPILLARY | PRECIPITATION — AGAR-GEL | COAGGLUTINATION |
|---|---|---|---|---|
| GROUP A | 76 | 100 | 100 | 100 |
| GROUP B | 21 | 100 | 100 | 100 |
| GROUP C | 31 | 100 | 100 | 96.8 |
| GROUP F | 13 | 100 | 100 | 100 |
| GROUP G | 29 | 100 | 100 | 100 |
| TOTAL | 170 | 100 | 100 | 99.4 |

TABLE 4.2

COAGGLUTINATION GROUPING AFTER 4, 6 & 20 HOURS OF INCUBATION COMPARED WITH CAPILLARY PRECIPITATION

| GROUPS | NUMBER DONE | PER CENT GROUPED: CO-AGGLUTINATION — 4H | CO-AGGLUTINATION — 6H | CO-AGGLUTINATION — 20H | CAPILLARY PRECIPITATION |
|---|---|---|---|---|---|
| A | 111 | 60 | 84 | 100 | 100 |
| B | 120 | 93 | 98 | 100 | 100 |
| C | 35 | 31 | 69 | 100 | 100 |
| D | 5 | 60 | 60 | 100 | 100 * |
| E | 22 | 32 | 46 | 100 | 100 |
| F | 117 | 47 | 75 | 100 | 100 |
| TOTAL | 410 | 62 | 82 | 100 | 100 |

* Precipitation grouping failed from THB culture for 2 group D strains but was successful when grown in glucose-phosphate broth.

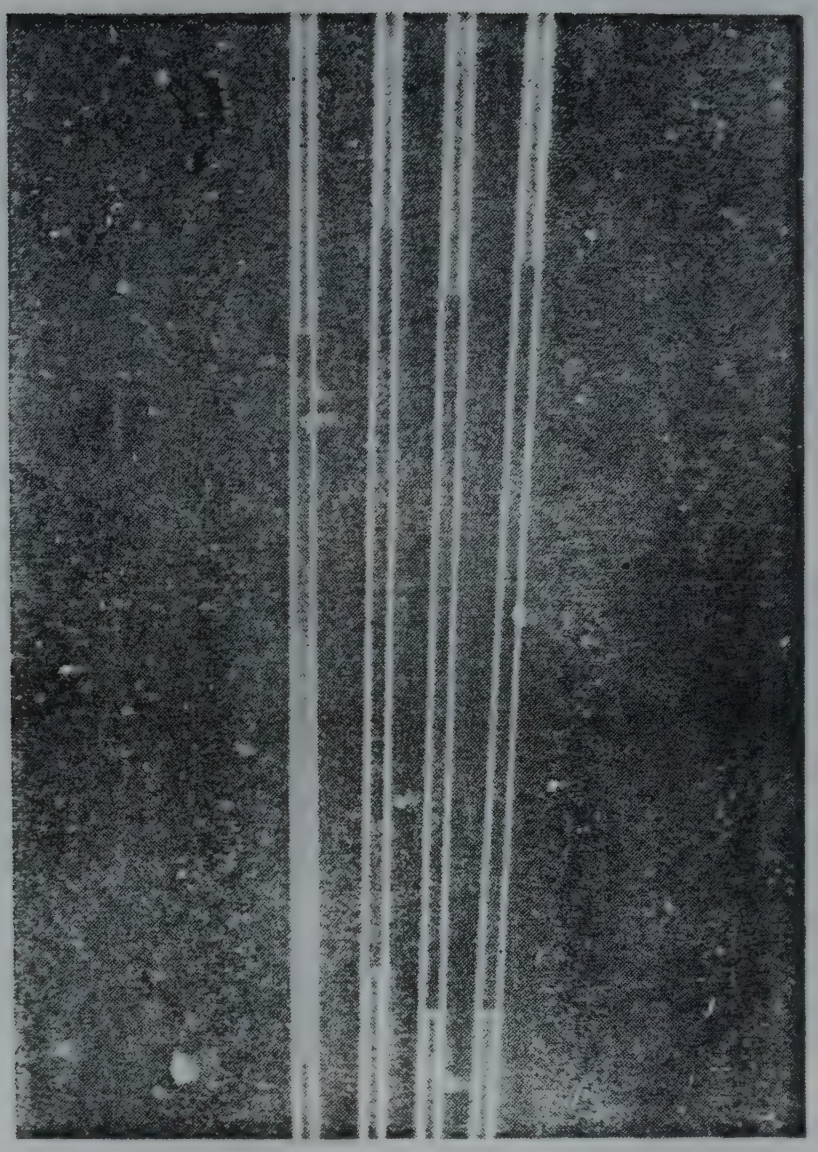


FIG. 4.1.

THE CAPILLARY PRECIPITATION METHOD

OF GROUPING STREPTOCOCCI

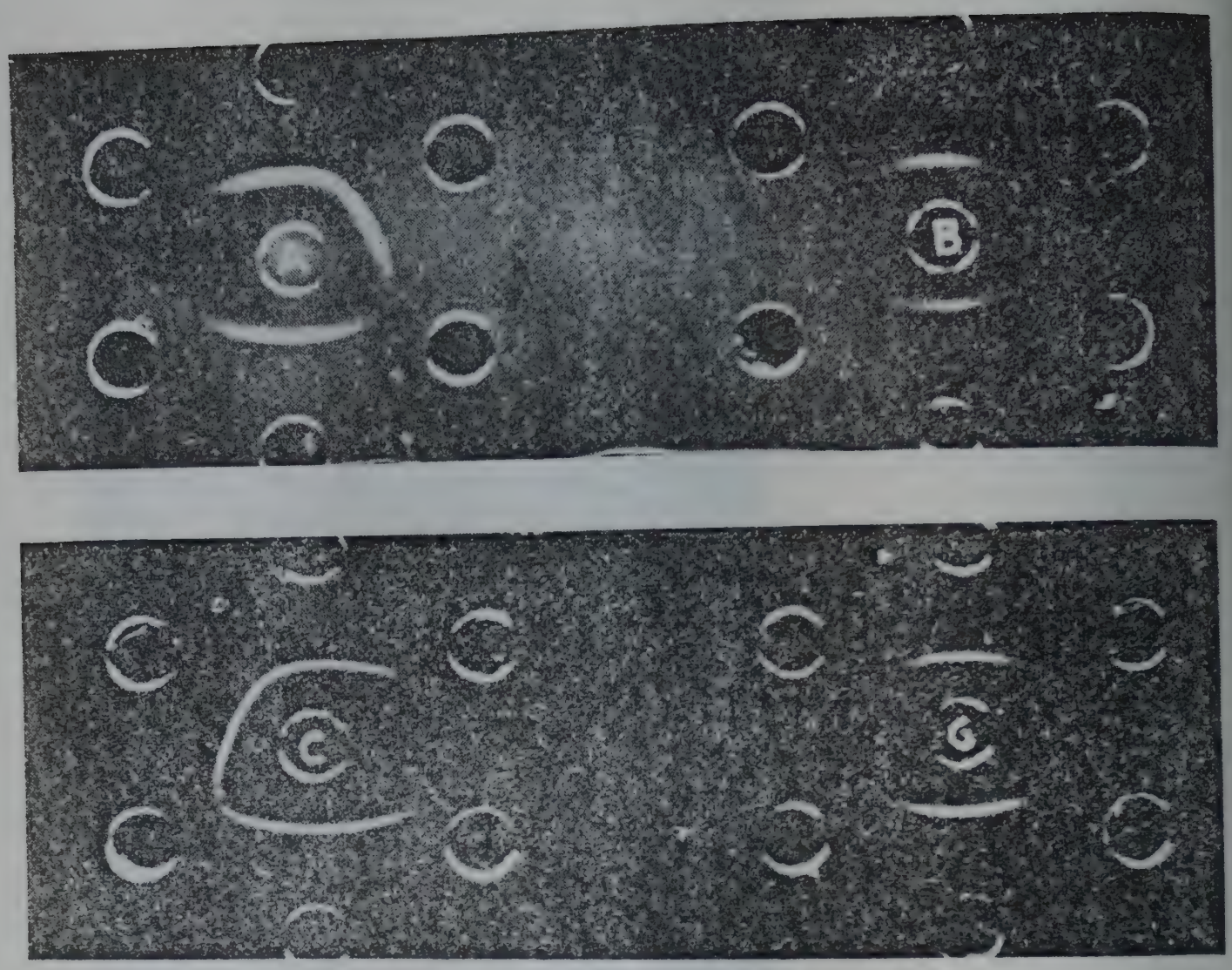



FIG. 4.2.

AGAR GEL PRECIPITATION FOR GROUPING STREPTOCOCCI.
NOTE THAT GROUP B PRECIPITIN LINES THOUGH
(DIFFUSE) AND FAINT ARE CLEARLY SEEN

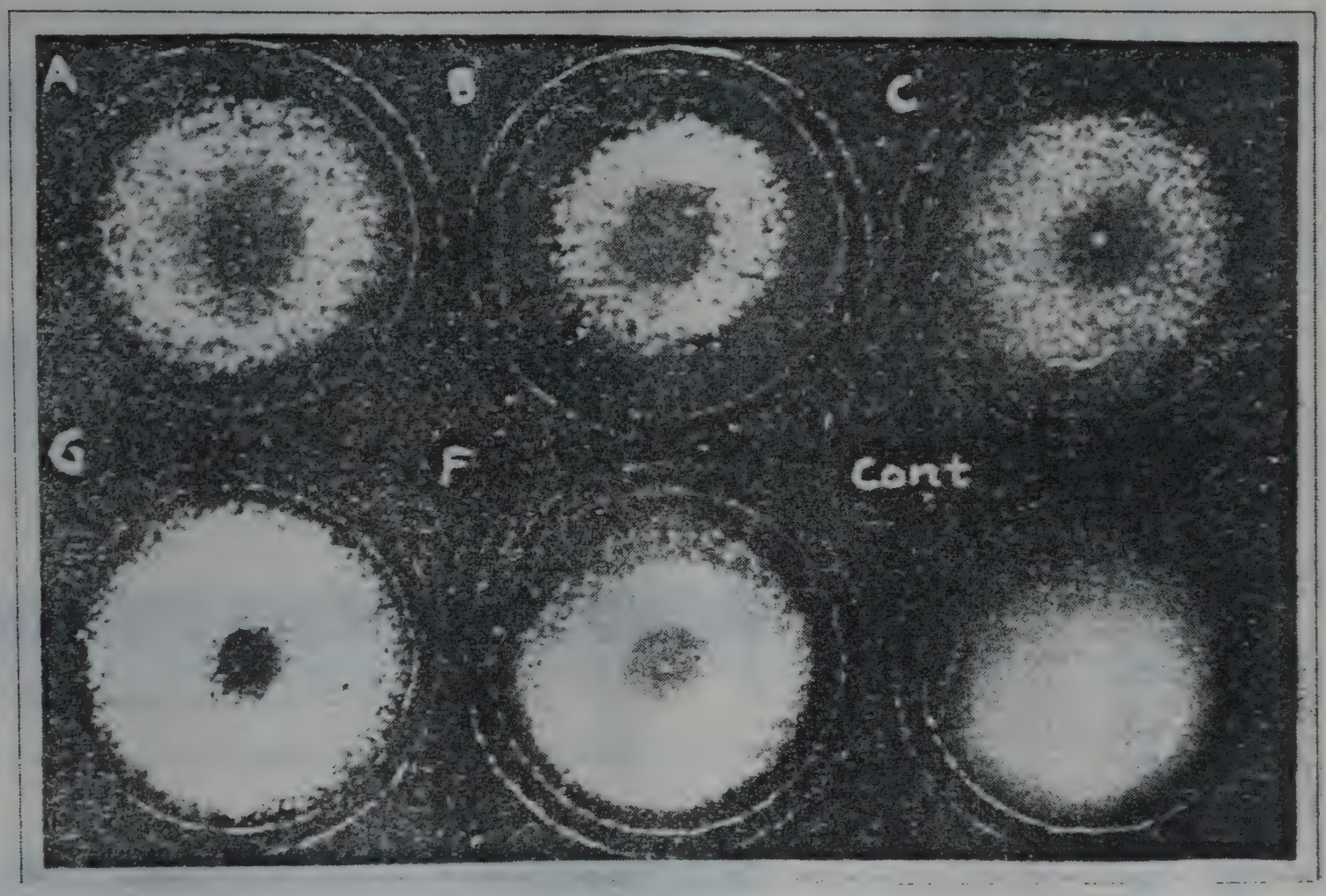
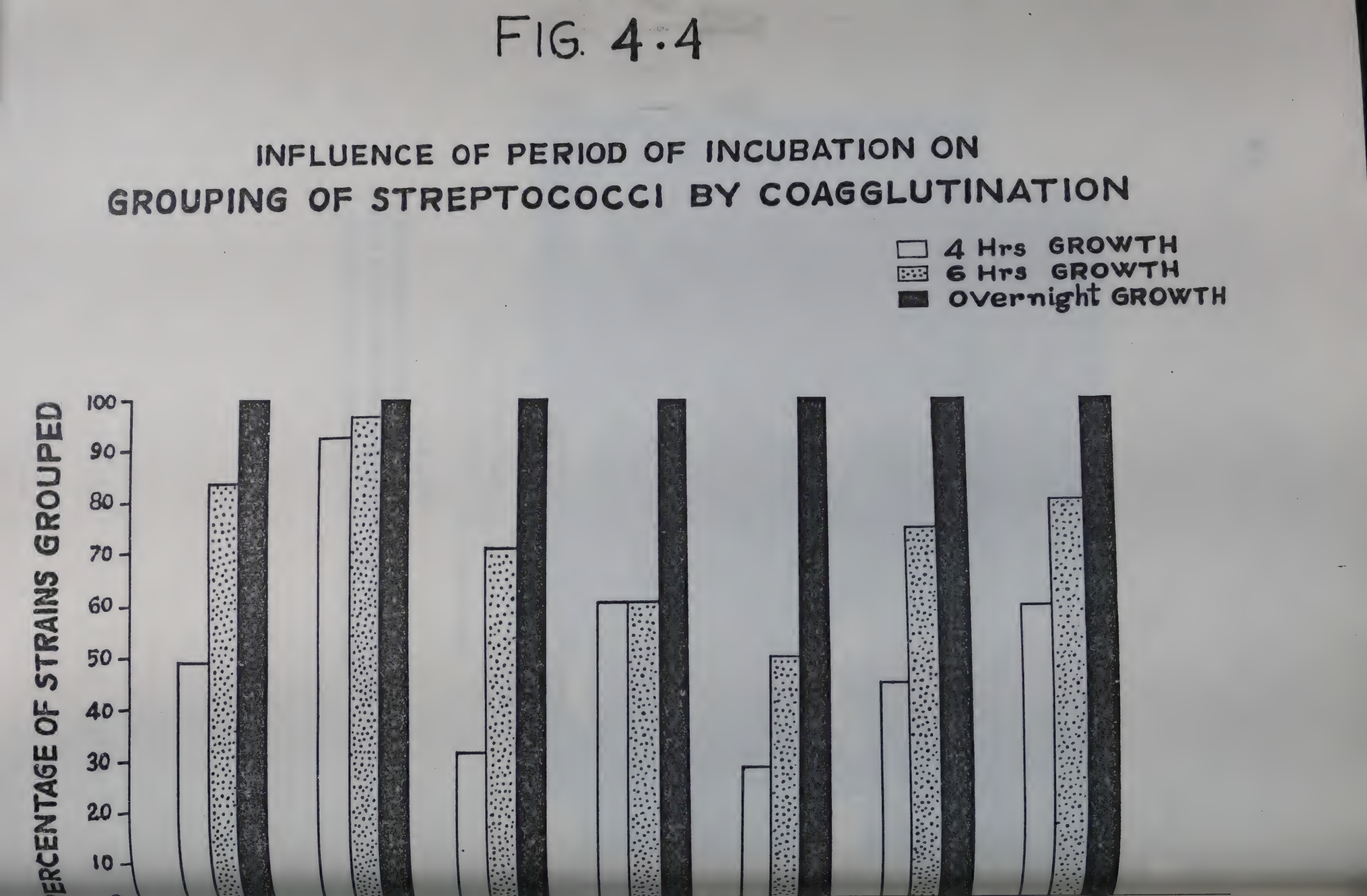



FIG. 4.3.

CO-AGGLUTINATION METHOD OF GROUPING STREPTOCOCCI
CO-AGGLUTINATION OF DIFFERENT ANTIGENIC GROUPS WITH
THEIR HOMOLOGOUS STAPHYLOCOCCAL REAGENT ARE SHOWN

FIG. 4·4

## INFLUENCE OF PERIOD OF INCUBATION ON GROUPING OF STREPTOCOCCI BY COAGGLUTINATION



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## CHAPTER 5

## CARRIAGE OF BETA-HEMOLYTIC STREPTOCOCCI IN RURAL SCHOOL CHILDREN IN VELLORE, SOUTH INDIA

It is important to have an idea on the normal carrier rate of beta-hemolytic streptococci (BHS) in the population, to understand the magnitude of the problem of streptococcal infections & their sequelae. Such a preliminary study was undertaken by Myers and Koshi[1] during a period of 8 months in 1957-58 among children, youth and adults, to correspond to different seasons. Of the 2536 throat cultures examined, their was a gross carrier rate of 35.5%, with 5% group A, 6% group B, 8% group C, & 13% group G streptococci. There was no consistent seasonal variation, though there was variation from one social group to another in particular seasons. The high carrier rate found in certain social groups might have been because of the sampling adopted, which included not only the healthy carriers but also those presumably "infected" as well.

In the present surveillance study, every attempt was made to include only those who were healthy, with no apparent sore throat, to get a baseline data on the normal carrier rate of BHS in order to delineate some epidemiological information, on the population included in the surveillance study.

## Material and Methods

Healthy carrier study was so adjusted to cover different seasons of the year in different schools. The three seasons in general, corresponded to "Winter" or "Cold" season (November to February), "Summer" (March to June), and "Rainy" (July to October) seasons.

Swabbings of the throat, skin & nose were done using nontoxic cotton swabs. Method of collection of swabs, transport of specimens, cultural procedures and identification were as given in Chapter 2.

## Results

The control study during "Winter" was limited to 111 in the last 5 schools - RS 12, RS 13, RS 14, RS 15, & RS 16 - during the 2nd survey in October to December 1976 and 121 in the 4th survey in RS 05 & RS 08 from December 1976 to February 1977, a total of 232 controls. The summer swabbings of controls in the first 10 schools, was in March & April 1976 and included throat & skin swabs of a total of 1643. The "Rainy" season controls included first survey (July to August 1975) in RS 05, RS 06, & RS 07 (567 throat swabs) in the rainy season of 1976 and the first survey of the last 5 schools RS 12, 13, 14, 15, & 16 (466 throat swabs) in the rainy season of 1976 ; the survey throat swabs in the above schools totalled 1033, & skin swabs 508. The third survey in the first 10 schools, done during July to Novermber 1976 included 1643 throat and skin swabs, giving a grand total of 2676 throat swabs & 2151 skin swabs during

rainy season from controls. Results on BHS are summarised in Table 1. Antigenic groups in different seasons are represented in Table 2. The bar diagram (Fig. 1) giving the BHS isolation rate with antigenic groups from different sites during different seasons gives a clear cut idea about the variation of BHS & different groups at different sites in the different seasons.

## DISCUSSION

There are widely varying results of BHS & ABHS carrier rate reported in the literature [1-4]. However, not only were the population different, but the criteria used for inclusion in different studies as well as techniques used, had differed. In some studies, only healthy children were included [3,5]. In others [1], children, youth and adults were included & swabbing was done in all, which obviously included some with sore throat. In 1965-1967 on hospital based study, Koshi & Myers [5] found, in 110 normal healthy children up to 15 years of age, a throat carrier rate of 34% BHS with ABHS, only 4%; results were not very different from that of Myers & Koshi [1]. However, in the pilot study, we undertook among rural & urban school children [6], normal, healthy, control children from different schools were swabbed.

In the preliminary survey carried out by Myers & Koshi [1] in 1957 - 1958 in a total of 2536 throat swabs, there was a gross BHS carrier rate of 35.5% with ABHS 5%. Among the 769 control children in the Pilot study [6], the throat carrier rate was 24% & ABHS 6.5%.

In the present more controlled study, in the group of healthy rural school children with no sore throat, there was a gross throat carrier rate of 17.5% BHS with 2.1% ABHS among the 4551 control children when all seasons were taken together. This gross throat carrier rate of BHS & ABHS was much lower than that found by Myers & Koshi[1] earlier. We had also found that the gross carrier rate of BHS in the normal controls was 17.2% in the throat, 5.1% in the skin, and 4.9% in the nose. There was a clear cut seasonal variation in all 3 sites. Highest carrier rate was in the winter (or cold season), namely 33.2% of BHS with 8.2% of ABHS in the throat, and a skin carrier rate of 15.1% of BHS, 12.9% with ABHS in the skin. The nasal carrier rate was the least even in winter - BHS was 7.8%; ABHS 2.5% (Table 1 and 2 & Figure 1).

Prakash et al[7] on the other hand, in a rheumatic fever and rheumatic heart disease prophylaxis programme, had obtained, among 240 healthy school children, a carrier rate of 34.6% BHS, 57.8% of isolates being ABHS, 24.1% GGS, 8.4% GCS. However, there is doubt on the selection of the school children in the above study & probably would have included both normal healthy controls as well as those with (uncomplaining) URI, which would have accounted for the very high ABHS carrier rate.

Interestingly enough, the high throat carrier rate of groups C & G in our previous studies[1,5,6] and in the present study was a consistent finding in all seasons. In summer

and winter, the throat carrier rate of groups C & G exceeded that of ABHS and varied from 4.5 to 9.9% of GCS in different seasons and that of Group G Streptococci (GGS) was 10.9% both in summer & winter. Thus the high throat carrier rate of GCS and the very high carrier rate of GGS was a consistent finding in the 1957 study[1], in the Pilot study[6] and in the present more controlled study at Vellore. These high rates of groups C and G also were found in the throat of pharyngitis patients (Refer Chapter 7) in the present study.

The significant finding of seasonal variation in normal carrier rate was a noteworthy finding in the present study. Similarly, the gross skin carrier rate of BHS was 5.1% with ABHS 4.4%. But the skin carrier rate during winter was 15.1% & ABHS 12.9%. Thus the skin carrier rate of ABHS was higher than that in the throat ; the importance of higher skin carriage of ABHS, is possibly one of the contributory epidemiological factors in the higher prevalence & incidence of streptococcal pyoderma in rural school children of South India. The gross nasal carrier rate of BHS was only 4.9% with ABHS 2.5% in winter.

Thus it is concluded that the base-line data on the normal carrier rate in the throat, skin & nose is a must, before interpreting results of any streptococcal infection. Besides, data on streptococcal antibody level determination also is a must in the confirmation of bonafide streptococcal infection.

TABLE 5.1.

SEASONAL VARIATION IN THE CARRIER RATE OF B.H.S. OF RURAL SCHOOL CHILDREN, VELLORE, SOUTHERN INDIA

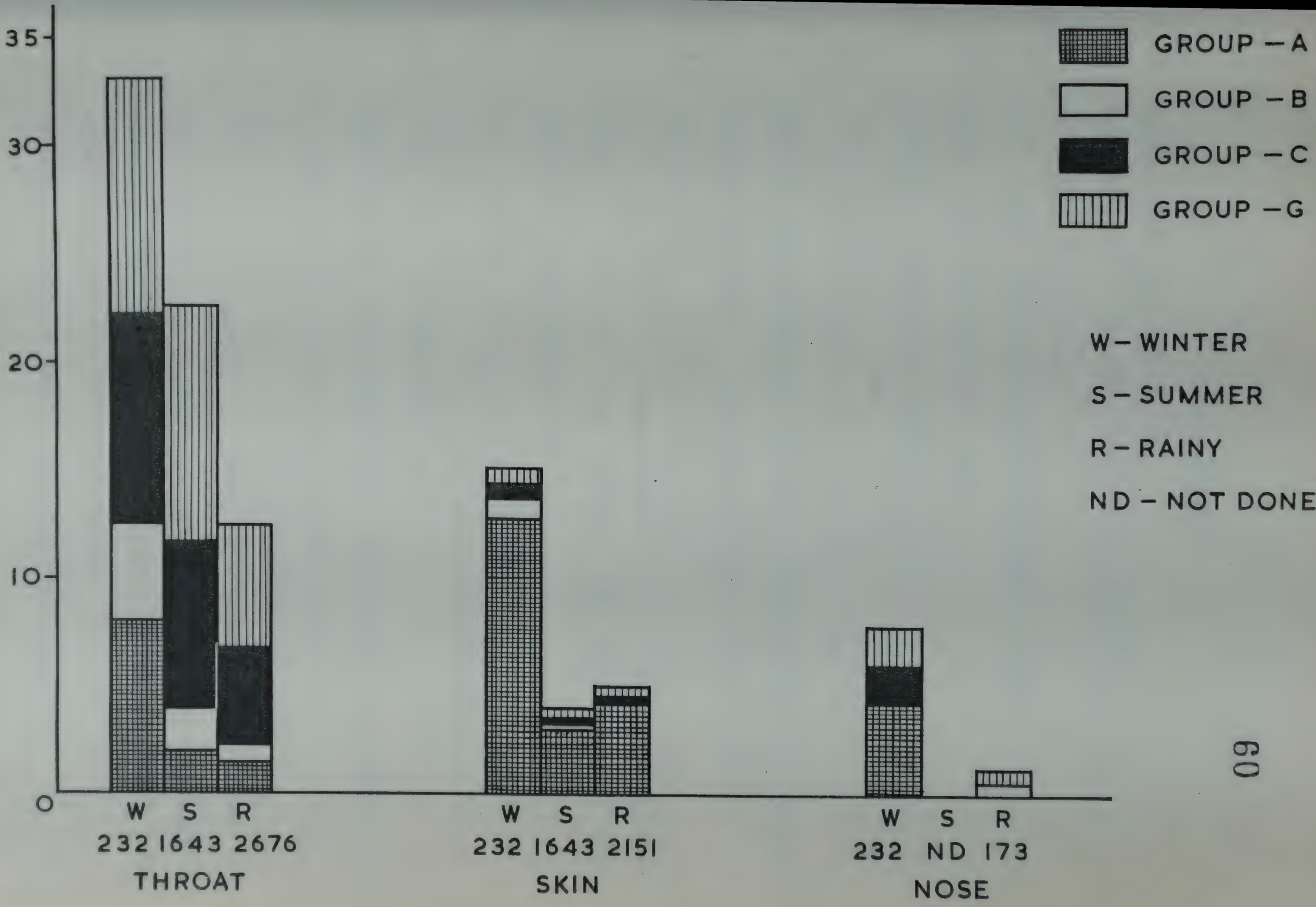
| SITE | SEASON | NUMBER DONE | B. H. S. NUMBER | B. H. S. % |
|---|---|---|---|---|
| THROAT | WINTER | 232 | 77 | 33.2 |
| | SUMMER | 1643 | 371 | 22.6 |
| | RAINY | 2676 | 337 | 12.6 |
| | TOTAL | 4551 | 785 | 17.2 |
| SKIN | WINTER | 232 | 35 | 15.1 |
| | SUMMER | 1643 | 67 | 4.1 |
| | RAINY | 2151 | 105 | 4.9 |
| | TOTAL | 4026 | 207 | 5.1 |
| NOSE | WINTER | 232 | 18 | 7.8 |
| | SUMMER | NOT DONE | | |
| | RAINY | 173 | 2 | 1.2 |
| | TOTAL | 405 | 20 | 4.9 |

TABLE 5.2

CARRIER RATE OF ANTIGENIC GROUPS OF STREPTOCOCCI

IN RURAL SCHOOL CHILDREN IN DIFFERENT SEASONS

| SITE | SEASON | BHS | GROUPS A No. | A % | B No. | B % | C No. | C % | G No. | G % |
|---|---|---|---|---|---|---|---|---|---|---|
| | WINTER | 77 | 19 | 8.2 | 10 | 4.3 | 23 | 9.9 | 25 | 10.8 |
| THROAT | SUMMER | 371 | 35 | 2.1 | 32 | 1.9 | 126 | 7.7 | 178 | 10.8 |
| | RAINY | 337 | 42 | 1.6 | 20 | 0.8 | 120 | 4.5 | 155 | 5.8 |
| | | 785 | 96 | 2.1 | 62 | 1.7 | 269 | 5.9 | 358 | 7.9 |
| | WINTER | 35 | 30 | 12.9 | 3 | 1.3 | 1 | 0.4 | 1 | 0.4 |
| SKIN | SUMMER | 67 | 53 | 3.2 | 4 | 0.2 | 6 | 0.4 | 4 | 0.2 |
| | RAINY | 105 | 94 | 4.4 | - | - | 3 | 0.2 | 8 | 0.4 |
| | | 207 | 177 | 4.4 | 7 | 0.2 | 10 | 0.2 | 13 | 0.3 |
| | WINTER | 18 | 10 | 4.3 | - | - | 4 | 1.7 | 4 | 1.7 |
| NOSE | SUMMER | NOT DONE | | | | | | | | |
| | RAINY | 2 | - | - | 1 | 0.6 | - | - | 1 | 0.6 |
| | | 20 | 10 | 2.5 | 1 | 0.2 | 4 | 1.0 | 5 | 1.2 |


35
30
20
10
0
GROUP – A
GROUP – B
GROUP – C
GROUP – G
W – WINTER
S – SUMMER
R – RAINY
ND – NOT DONE
W S R
232 1643 2676
THROAT
W S R
232 1643 2151
SKIN
W S R
232 ND 173
NOSE

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# CHAPTER 6

# STREPTOCOCCAL ANTIBODY IN HEALTH AND VALUE OF MULTIPLE ANTIBODY TESTS

Antibodies to various extracellular products of Group A streptococci are known to be produced in acute streptococcal infections of the throat and the skin. Those which have been studied, following natural infection in humans, include antibodies to streptolysin O, streptokinase, hyaluronidase, deoxyribonucleases and nicotinamide adenine dinucleotidase. The determination of these antibody titres is of value in the diagnosis of rheumatic fever (RF), rheumatic heart disease (RHD) as well as post-streptococcal glomerulonephritis (PSGN) [1-4].

Because of the ubiquity of streptococcai infections, sera from most normal individuals show some antibody to the various extracellular antigens of group A beta-hemolytic streptococci (A-BHS) [5-7]. The antibody response against the various strepto-coccal antigens depend on the site of infection, the nature of the sequelae which follow streptococcal infections and also by the type of test used to detect the antibody [2,8].

## MATERIALS AND METHODS

Sera from normal healthy school children, as well as those with pyoderma, were tested for anti-streptolysin O (ASO), anti-hyaluronidase (AH) and anti-deoxyribonuclease B (ADNB). Sera of healthy adult volunteers and hospital patients without streptococcal infection or sequelae were also tested.

The ASO test was carried out by microtiter technique as reported by koshi[9]. In the early part of the present study, the streptolysin O (SLO) antigen, ASO standard serum, etc. were purchased from Difco Laboratories, U.S.A. Later, these reagents were produced in this laboratory and standardised by methods recommended by the World Health Organization (Appendix IV). The AH test was carried out by microtiter technique[10] using commercially available reagents from Difco, U.S.A. The ADNB titration was carried out by a micromethod, similar to the one described by Nelson *et al*.[11]. The purified deoxyribonuclease B enzyme was prepared and standardised in this laboratory with the help of Dr. Rotta, WHO Consultant, Prague, Czechoslovakia (Appendix IV).

## RESULTS

Sera from only 25 to 33 healthy children could be obtained for titration of ASO, AH and ADNB as there was resistance from school children and their parents for collection of blood for serological studies. The results

are given in Fig. 6.1. The geometric mean titre (GMT) of ASO, AH and ADNB in this group of school children aged 5-15 years were 235.4, 350.9 and 811.9 units respectively. The GMT for AH and ADNB titres of healthy adult volunteers and children without streptococcal infection seen at the CHRISTIAN MEDICAL COLLEGE HOSPITAL at different times are given in Fig. 6.1a. The GMT of AH for 30 children aged $\leqslant$ 15, years was 235.6 units and for 75 adults, it was 201.2 units. Similarly, the GMT of ADNB titres of 29 children aged $\leqslant$ 15 years seen in the hospitals was 401.2 units and for 135 adults it was 358.8 units.

For ASO, the results of studies on similar control children carried out earlier [14], were adopted, i.e. titres of 240 units and 340 units were taken as significant for children $<$ 5 years and $\geqslant$ 5 years respectively.

Based on the results of antibody determinations in children and adults seen in the hospital, titres of 1024 units and 800 units were taken as significant titres for A H and ADNB respectively.

ASO, AH and ADNB titres in 220 school children with pyoderma, who yielded group A streptococci from their skin lesions are depicted in Fig. 6.2 as a scattergram.

Based on this, the percentage of elevated titres of ASO, AH and ADNB in children with pyoderma, as well as combinations of one or more of these antibody titres are

depicted in Fig. 6.3. Significant ADNB titres were seen in 90 percent of children with proved streptococcal pyoderma,. whereas significant AH and ASO titres were seen only in 66% and 40% respectively. Significant titres of all three antibody tests was observed in 95% children. By employing a combnination of AH and ADNB or ASO and ADNB 93% of children with elevated antibody titres could be detected. Tests for ASO and AH revealed elevated titres in only 76 per cent of children.

## DISCUSSION

An elevated or 'significant' antibody titre against streptococcal extracellular products is not in itself diagnostic for RF, RHD or PSGN. However, when combined with clinical findings, it is of great value in arriving at a diagnosis because it documents the streptococcal infections which precede these sequelae. To assess the `significant' or elevated titres of antibodies to individual streptococcal antigens, it is necessary to determine these antibody titres in control subjects who are free of current or recent streptococcal infections. The levels of the different streptococcal antibodies vary in different populations and are influenced by age, season, year overcrowding, geographical area, site of infection and other factors related to the frequency of streptococcal infection [2,8,12].

Although it is ideal to test two samples of sera from patient, i.e. one collected during the acute phase of the illness and the other during the convalescent phase, to study a `rise' in antibody titre, it is not always practicable. Moreover, patients with sequelae are usually not seen early enough to obtain both acute and convalescent sera. In this situation, a `significant' or `elevated' titre based on the antibody levels in normal control subjects can be used for determining the significance of streptococcal antibody titres.

For practical purposes, two working dilutions above the GMT value in control subjects is taken as significant or elevated titre[13]. Such elevated or significant titres are usually seen in about 20% of normal healthy individuals. By calculating the GMT for ASO titres in a group of 110 control subjects in Vellore, it was found that the titres of 240 units and above can be taken as significant for children through 5 years of age, and titres of 340 units and above as significant for children in the 6-15 year age group, as well as for adults[14,15]. Significant titres of ASO, for different populations, reported in the literature vary from 160 units[16] to 340 units[5,17].

The GMT of ASO titer among 33 control rural school children was 235.4 units and hence the significant or elevated titres for this population should be 480 units and above. This high level of significant ASO titre was

probably becaues of an outbreak of streptococcal infection in this population, just before the survey. Further, infection with Group C streptococci can also result in elevated ASO titres[18]. Hence, data based on this group of children were not considered for determination of significant titres in the infected group, in the present study. The same reasons were found to hold good for ADNB titre also. Reported significant levels of ADNB titre ranged from 250 units[19] to 400 units[17]. Unlike ASO which is not usually elevated after streptococcal infections of the skin, ADNB levels rise to vary high titres after skin infections[20]. Moreover, ADNB titres tend to remain elevated for a longer period of time[21]. The high level of significant titer, i.e. 1600 seen in the present study among rural school children (Fig. 6.1) is more probably because of the high level of endemicity of A-BHS pyoderma (Refer Chapter 8 of this report) in this population. However, based on ADNB titers done in hospital volunteers, (Fig. 6.1a) titers of 800 and above were considered elevated, both in children and adults. Because of the above facts, the significant levels established for ASO and ADNB on hospital based controls were taken as base line data for the present study also.

The significant titre of AH reported in different studies vary from 128 to 1024[21,22]. In the present study the GMT of AH in hospital children and healthy adults were 235 units and 201 units respectively (Fig. 6.1a). However, the

GMT of AH in 33 healthy school children was 351 units(Fig.6.1). Like ADNB, AH titres seem to be better indication of streptococcal skin infections than ASO.

## Value of multiple antibody tests

It has been shown that by testing for more than one antibody in patients with streptococcal infections and their complications a high percentage of patients can be shown to have serological evidence of previous streptococcal infection [4,23]. Our results on ASO, AH and ADNB titres in 220 school children with culture proved streptococcal pyoderma, has revealed that by using these 3 tests 95% of infected pupils could be shown to have an elevated antibody titre against at least one streptococcal antigen (Fig.6.2).

As expected, ASO was elevated in only 40% of children with streptococcal pyoderma, whereas AH and ADNB were elevated in 66% and 90% respectively. Testing for either ASO and ADNB or AH and ADNB revealed elevated titres in 93% of children with pyoderma, whereas ASO and/or AH were elevated in 76 percent only.

Multiple antibody tests were rewarding in confirmation of streptococcal pharyngitis also as is given in Chapter 7 (Table 7.5,7.6) as well as in RF & RHD as given in Chapter 10.

Thus, our study confirmed the value of ADNB and/or AH titration besides ASO for maximal serological confirmation of streptococcal infections.

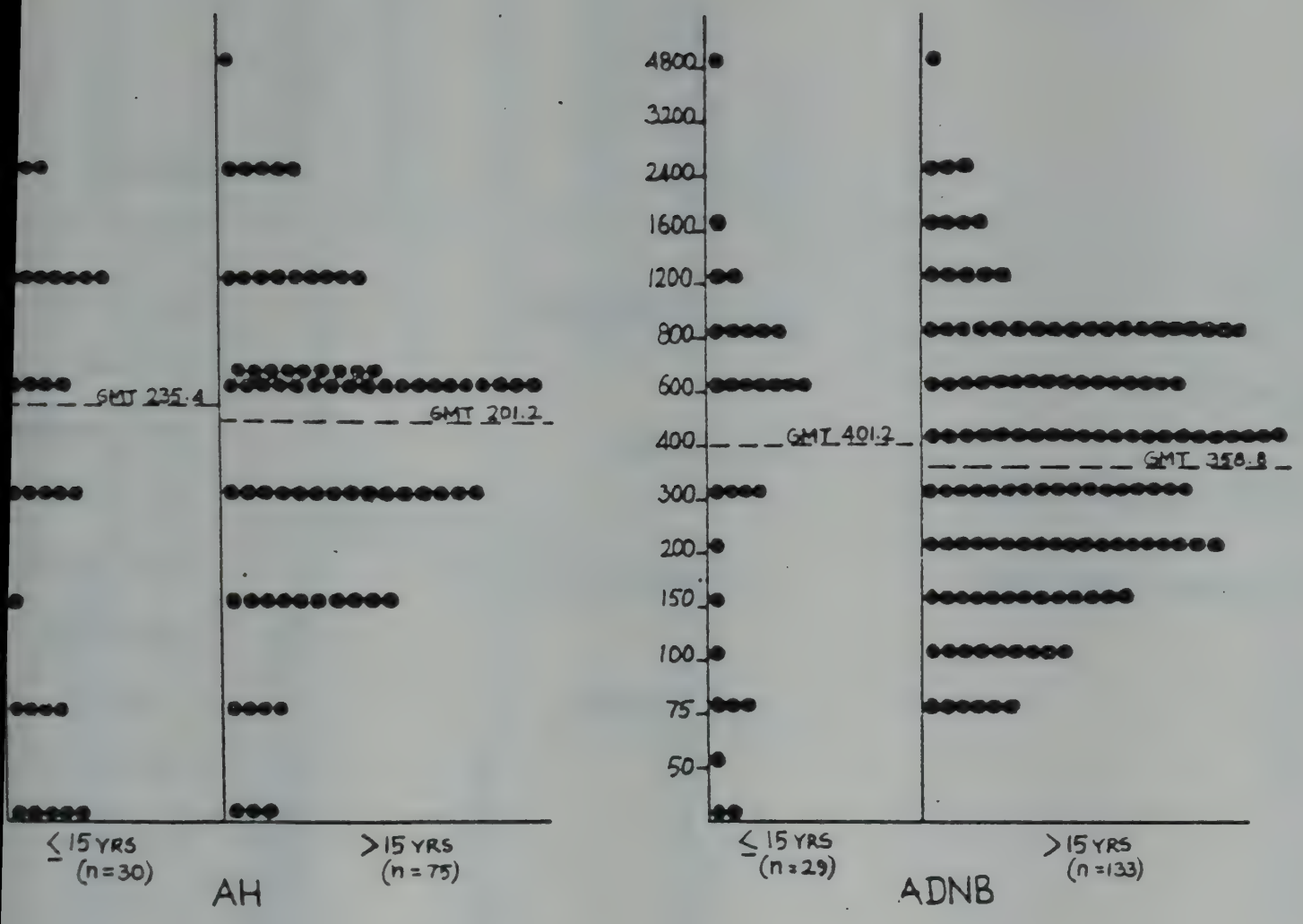



FIG 6.1a

AH AND ADNB TITRES IN CONTROLS

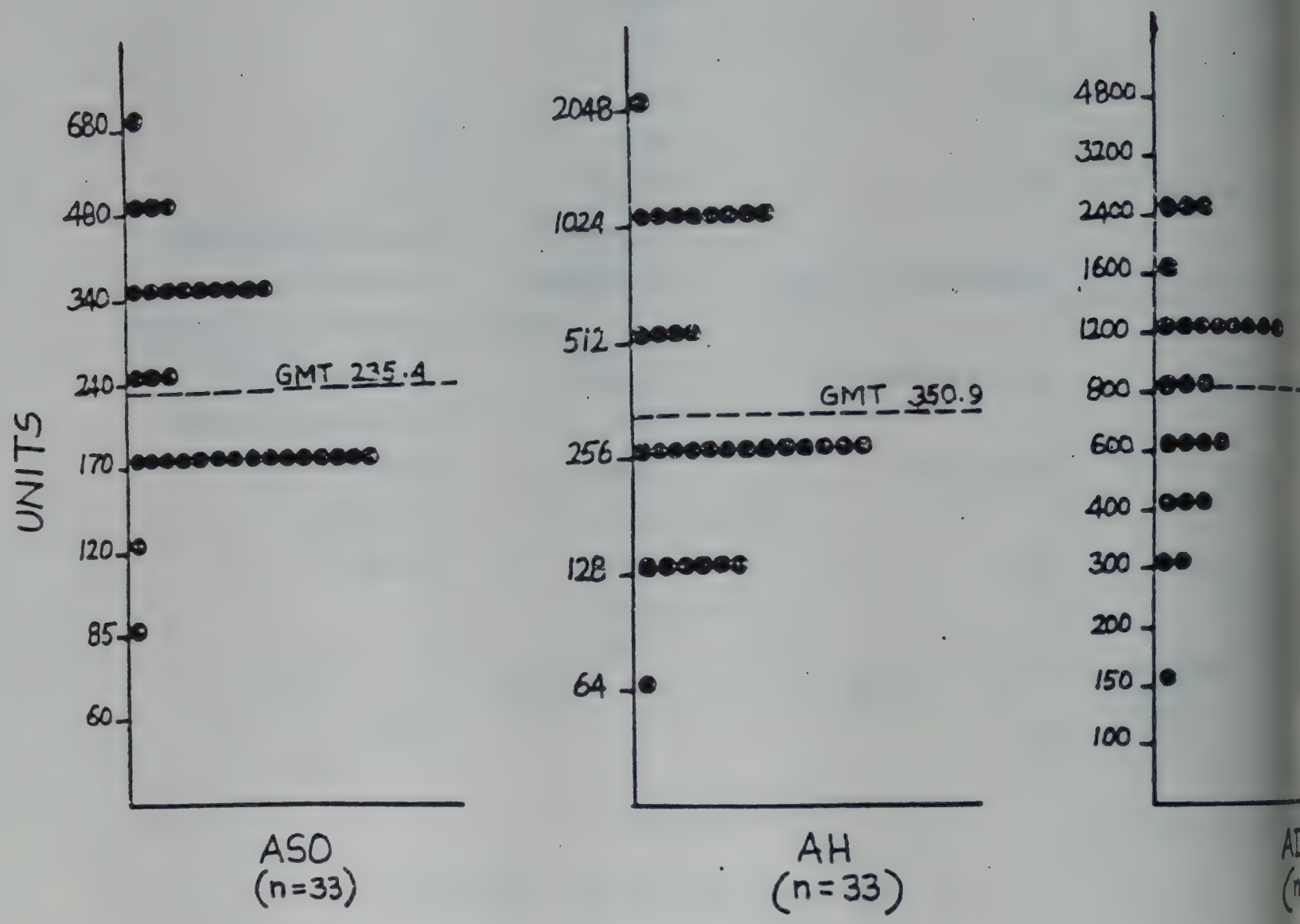



FIG. 6.1

STREPTOCOCCAL ANTIBODY TITERS IN HEALTHY SCHOOL CHILDREN

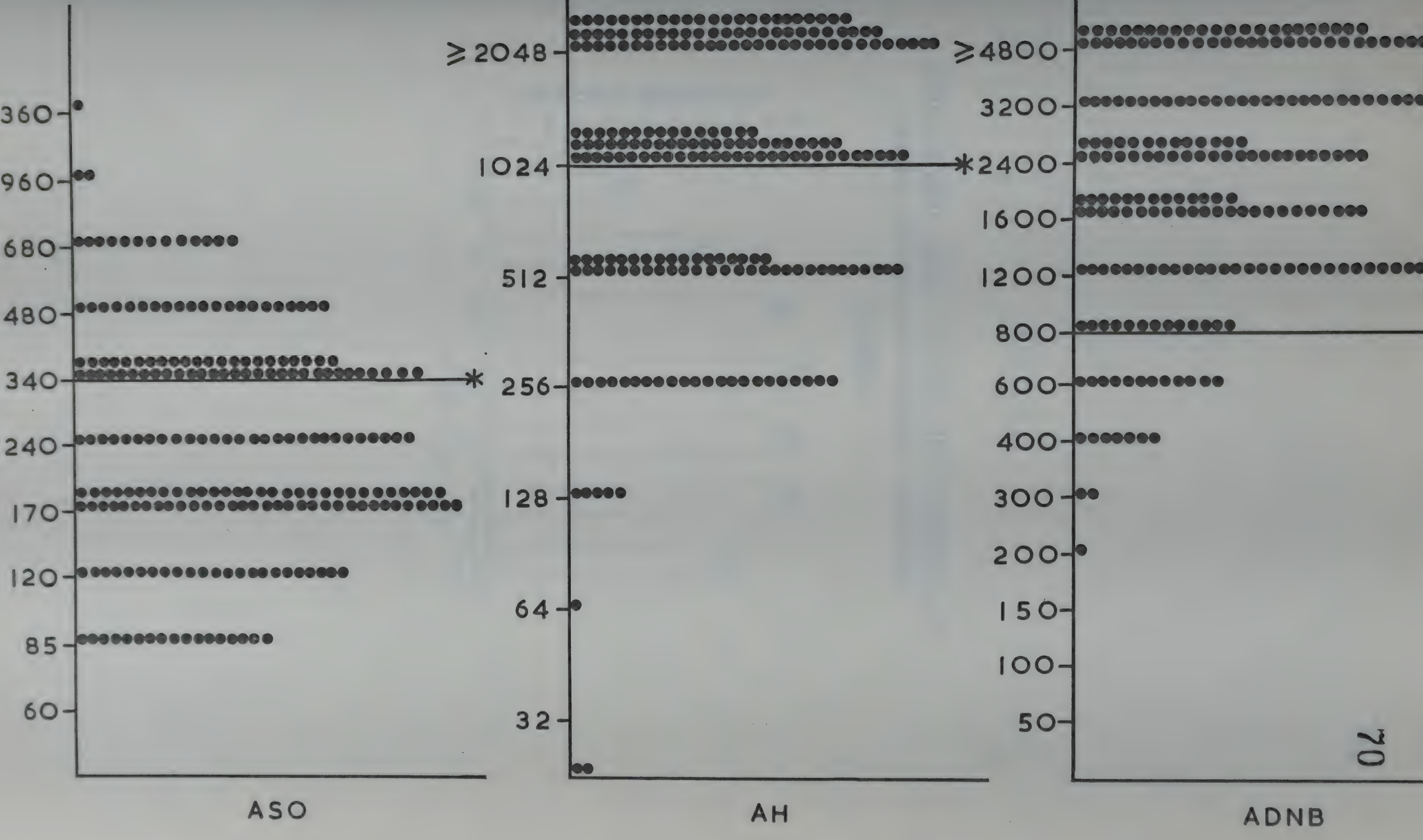
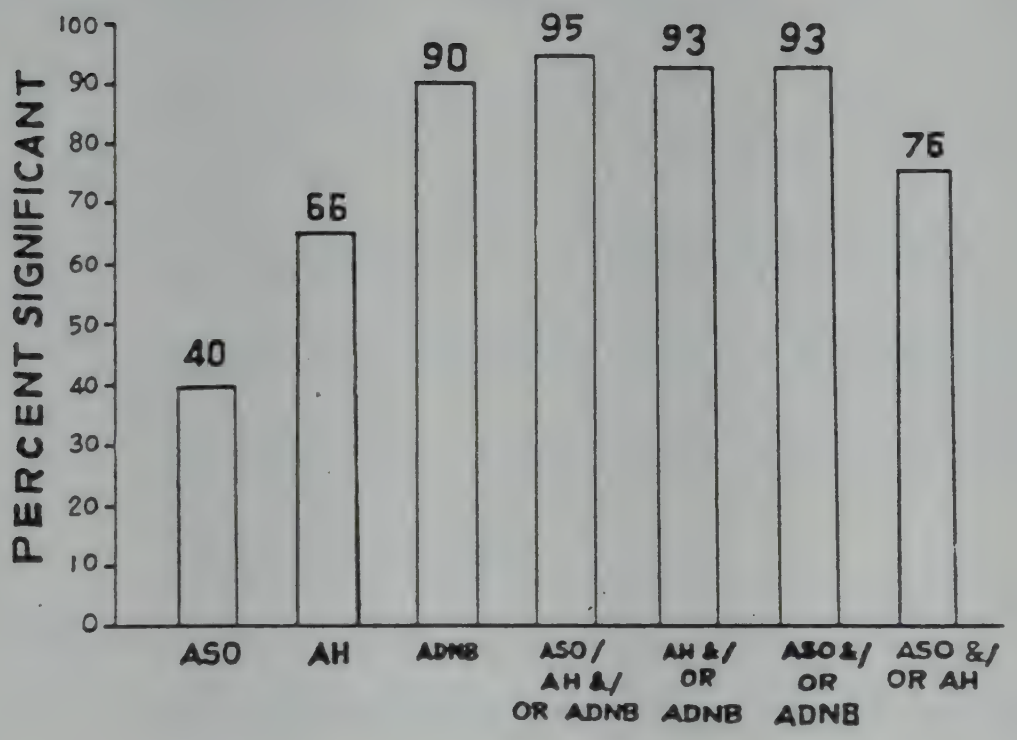

UNITS
1360
960
680
480
340
240
170
120
85
60
ASO
≥2048
1024
512
256
128
64
32
AH
≥4800
3200
2400
1600
1200
800
600
400
300
200
150
100
50
ADNB
70

FIG 6.3

ASO, AH AND ADNB TITRES IN PYODERMA OF SCHOOL CHILDREN WITH GROUP A ISOLATION

(n=220)



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# CHAPTER 7

## STREPTOCOCCAL PHARYNGITIS AND ITS CONTROL IN RURAL SCHOOL CHILDREN

Streptococcal pharyngitis is a term used interchangeably for tonsillitis, tonsillopharyngitis, pharyngitis, nasopharyngitis upper respiratory infection, etc., and here after refered to as Pharyngitis. Group A beta-hemmolytic streptococci (ABHS) is responsible for producing not only pharyngitis but also rheummatic fever (RF) and post-streptococcal glomerulonephritis (PSGN). Streptococcal pharyngitis is a disease found predominantely in schools. It has been rightly refered to as an "Occupational disease" of school children. The incrimination of ABHS in the genesis of RF and in the repeated recurrences of rheumatic heart disease (RHD), as well as in causing acute nephritis, has made the organism a unique pathogen.

Streptococcal infection of the throat is a world-wide problem. However, its incidence varies in different countries of the world and in different parts of the same country[1]. All cases of clinical pharyngitis are not streptococcal. Though often a sore throat is taken as streptococcal pharyngitis by most people, a large majority are of viral etiology[2]. Bacteria such as S.Pneumoniae, H. influenzae and others can also be occasionally involved in pharyngitis. Streptococcal carriers of the throat and nose

are to be differentiated from those with active infection and the carriage varies from country to country[1,3]. Streptococcal carriers are those who harbour the streptococci in the throat or nasopharynx without revealing any evidence of recognising its presence by a "rise" in the titre of streptococcal antibody. Streotococcal carriers need not be treated, unless indicated in special circumstances.

The clinical symptoms of streptococcal pharyngitis include sudden onset of fever (about 101°F to 104°F) with sore throat, dysphagia and in some children, cough, abdominal pain, nausea and vomiting may be prominent. Signs of anterior cervical lymphadenits, rhinitis, red throat with exudate and enlarged tonsils are more often associated with ABHS isolation[4]. It is very important that streptococcal pharyngitis is confirmed by microbiological investigations before treatmennt is instituted. Since in 'bonafide' streptococcal innfection, besides the presence of ABHS in the throat or nasopharynx, the person reacts to its presence by an antibody "rise", it is important that both nasopharyngeal cultures as well as ntibody determinationn in paired samples of sera are donne for establishing 'bonafide' streptococcal infection. Several workers have found that among those with pharynngitis and group A isolation, only about half show evidence of antibody rise and are thus truly infected.

The persistent interest in streptococcal pharyngitis is because of the possibility of preventing RF and PSGN if the primary streptococcal infectionn is controlled in time. The effectiveness of prophylactic administration of penicillin in preventing primary attacks of RF and recurrence in RHD has been demonstrated by various workers all over the world [5-9].

Streptococcal control programmes wherever undertaken have been successful in lowering the infection rate and in the control of RF and PSGN [10-14]. The need for proper programming, which is simple enough to follow, proper microbiological confirmation of streptococcal pharyngitis and regular follow up and low cost of operation has been stressed by most workers.

A pilot survey on streptococcal infections undertaken by us revealed the rarity of streptococcal pharyngitis proved by culture and serology as againist streptococcal pyoderma in South Indian children [15]. The pilot survey revealed the need for a long term surveiliance and control of streptococcal infection and sequelae. Hence such a surveillance of streptococcal infection was carried out from 1975 to 1978 among children belonging to 15 rural schools in southern India. A report on the findings of streptococcal pharyngitis in rural school children in the surveillance study undertaken is given here.

## MATERIAL AND METHODS

This has been detailed in Chapter 2. In brief, 15 schools belonging to 13 villages in Kaniyambadi block, North Arcot District, about 15 kilometers from the Christian Medical College Hospital, Vellore were included in the study. Each school was visited every 3 to 4 months, at least thrice during a school year excluding seasonal holidays. Preliminary informations were obtained by field workers during regular visits. Each child was seen, history clarified and clinical examination done by a clinician within one to two days of the original visit. Each pupil had a member card (Appendix I) filled in for all relevant points of history and findinds. Children with a history and/or findings of pharyngitis were examined in detail and the Pharyngitis Card (Appendix I) was filled in for such. Every attemmpt was made to see those who were absent, sometime during the same week.

From all children suspected of pharyngitis, throat swabs were taken by vigorously swabbing the tonsills, post-tonsillar fossa and posterior pharynx; care was taken to swab the follicles and to include exudate if any. Whenever the child cooperated, nasal swabs and blood for antibody titration were also obtained.

In the early part of the study, multiple methods of transportation and culture to recover BHS were carried out [16]. Since that earlier study proved the efficacy and superiority of the filter paper (FP) technique of transportation (Refer Chapter 3) the same was followed for the later studies. The FP was cultured on 5% sheep blood agar and was incubated at 35°C ± 1°c for 4 hours; it was transferred to the other half of BA and further incubation continued overnight. Beta-hemolytic streptococci were identified and groups and serotypes determined [17-20] (Refer Appendix III).

About 5 ml of blood was collected for anitbody estimation. For part of the study, the finger prick method was tried for ASO and it was compared with blood collected from venipuncture [21]. Whenever blood was adequate, anti-streptolysin O (ASO), antihyaluronidase (AH) and anti-deoxyribonuclease B (ADNB) were titrated on blood collected by venipuncture.

Children who were proved to have streptococcal pharyngitis, either by ABHS isolation (as serology could not be obtained in many) or by significant streptococcal antibody, were given primary prophylaxis with one injection of Benzathine Benzyl Penicillin 600,000 units I.M. if below 5 years and 1,200,000 units I.M. if above 5 years.

## RESULTS

The villages were given the code number preceeded by 'V' and schools were given the code 'RS'. The number of pupils varied in the two higher secondary schools and 13 primary schools covered by the surveillance programme. Among the two high schools, RS03 had 415 and RS05 had 606 pupils registered in the first survey. The male-female ratio and the age-group of those registered for the first survey in the 15 schools are given in Table 7.1. The male-female ratio was 3:2. There were only 278 (10.8%) in the 4 to 5 age-group, 1558 (60.5%) in the 6 to 10 and 741 (28.8%) in the 11 to 15 age-group. The attendance in the subsequent surveys, II to the IX, waxed and waned an varied from 83 to 104% from the II to the VIII survey (Table 7.2). The IX survey could not be carried out in the last 5 schools, RS12 to RS16, as the holidays interfered. With the exception of one survey, the overall attendance was above 92% and this percent was maintained because of new entries, inspite of dropouts. Inspite of our attempts, only 923 (35.8%) out of the originally registered 2577 childrenn attended all the 9 surveys.

In suspected pharyngitis, proper swabbings were done prior to any antibiotic therapy and the FP technique which was proved previously to be good for field study[16] was used; proper and adequate culturing techniques were carried out. Table 7.3 gives the results of throat cultures from all children with pharyngitis in the different surveys. The

prevalence of culturally proved pharyngitis in the 2577 children from the 15 schools during the first survey is represented in the bar diagram arranged in the frequency of isolation of BHS and ABHS (Fig.7.1). In 923 children who attended all surveys conducted, the frequency of isolation of BHS and ABHS from cases of pharyngitis is represented in Fig. 7.2 and in Table 7.4. It is seen that the isolation of BHS from the 2577 children of the 15 schools during the first survey was 3.1% and ABHS 0.9% (Table 7.3). Among the different schools RS10 had the maximum isolation of BHS 14.5% and ABHS 4.8 (Fig 7.2). In general the results in both the groups namely 2577 children seen during first survey and the 923 seen in all surveys were comparable (Fig. 7.1 and 7.2). Children of schools 01,02,13,15 and 16 did not yield any ABHS (Fig. 7.1). Among the 923 children seenn in all surveys children of 06,08,12 and 14 did not yield any BHS from throat cultures during the first survey.

As most of the children refused collection of blood for streptococcal antibody tests, only 23 had complete serology done and results are presented in Table 7.5. Any one antibody elevation was revealed in 91.3%. Multiple antibody tests, anti-streptolysin O (ASO), anti-hyaluronidase (AH) and anti-deoxyribonuclease B(ADNB) done on larger but varying numbers of sera revealed that ASO was elevated in 35.0% AH in only 40% and ADNB in 86.2% (Table 7.6). Thus ADNB was found elevated in 86% and was a better single test

in streptococcal pharyngitis as was in other diseases. Many of the suspected cases may have been viral.

Table 7.7 shows that 44% to 100% of children with streptococcal pharyngitis accepted prophylaxis during different surveys (these may have included some carriers).

The incidence (point prevalence) of streptococcal pharyngitis with BHS and ABHS recovery in the 923 children seen in all surveys conducted, is given in Fig. 7.3. The antigenic groups of total streptococci recovered from the throats of all children with suspected pharyngitis among the 923 seen during the 1st to 9th surveys is given in Fig.7.4. The incidence percent (point prevalance) of BHS and ABHS showed a descreasing trend through out the study. Culturally proved pharyngitis cases decreased in the latter surveys and in the last 4 to 5 surveys, few or none of the patients with pharyngitis had ABHS or BHS in the throat. Children of schools 11,12,13,14,16 did not have isolation of ABHS.

## DISCUSSION

The importance of streptococcal pharyngitis is mainly because of the development of post-streptococcal sequlae. During epidemics in military populations, it has been estimated that about 3% of untreated streptococcal infections are followed by rheumatic fever[22]. Attack rates following sporadic infections are lower but vary from country to country. Early and adequate treatment with

penicillin will eliminate streptococci annd prevent most attacks of RF. However, it is immportant to differentiate infection from carrier state and to administer proper treatment.

A "bonafide" infection is the condition in which not only group ABHS in recovered from throat cultures, but also where evidence for recognition of the presence of ABHS in the throat is revealed by a "rise" in titre of antibodies to streptococcal antigens[23].

The limitations in our study were that repeat cultures could not be done and most of the rural school children refused to give blood either by venipuncture or finger prick. There were instances where the whole team was driven out of the school and villages when the parents came to know that blood was taken from the children. Thus there was no way of difinitely confirming 'bonafide' streptococcal pharyngitis in our study. This problem has been highlighted by most workers in India. Hence, the results presented here are based on beta-hemolytic streptococci and group ABHS isolation in culture. As such, carriers of ABHS also may bave been included.

A total of 2577 children from 15 schools were registered in the study. The prevalence of ABHS pharyngitis in different schools varied from none in some schools to a maximmum of 4.8% in RS10 with an average of 0.9% ABHS in the population studied. This prevalence rate is lower than reported elsewhere and if the carriers were to be eliminated

by antibody studies, the prevallence rate would have been still lower. However, it is note-worthy that groups other than group A were encountered in children of all schools, and in some schools non ABHS to the exclusion of ABHS was noted to be predominating. The total BHS recovered varried from none to 14.5% with an average of 3.1% during the 1st survey (Table 7.3). The normal carrier rate of group G and C in the population in south India has been found to be very high at different sudies conducted [3,8,24].

Although group A is the most commonly responsible agent among the beta-hemolytic streptococci causing pharyngitis, other Lancefield groups have been known to cause similar clinical illness [25,26]. Isolated non-epidemic cases of pharyngitis have been associated with groups C and G and also with group B [2,27-31].

The results of the long term surveillance programme had its impact on streptococcal pharyngitis and BHS carriage in the population. In each of the 15 schools, the gradual or sudden elimination of the BHS and ABHS from the throat culture of children with clinical pharyngitis is evident (Fig. 7.3) in the various surveys conducted in 923 children who attented all the surveys. The point prevalence rates calculated for the varying number of children attending the schools at different surveys also showed a similar trend (Table 7.3 and Fig. 7.4).

The above results, as expected, emerged from the impact of the primary prophylaxis programme undertaken in the surveillance study. All the children with culture and/or serologically proved streptococcal pharyngitis were given inj. Benzathine Benzyl Penicillin withinn 3 days to a week. The primary prophylaxis undertaken had its impact in reducing the streptococcal pharyngitis, and the streptococcal load (carriage) in the student population. More important was that the main aim of the study namely, to control rheumatic fever by primay prophylaxis, was achieved. Not a single new case of RF or RHD was detected throughout 1978[9]. For details refer Chapter 10 of this report.

In summary, it may be emphasized that following important points emerged out of this study:

1) The prevalence of streptococcal pharyngitis in the rural community of children is minimal amd much lower than streptococcal pyoderma (Refer Chapter 8 of this report).

2) Non-Group A pharyngitis was also common as was the non-group A carriage rate( Refer Chapter 5).

3) The incidence (point prevalence) of streptococcal pharyngitis continued to decline as the surveillance programme was accepted by the community with increasing enthusiasm. There were no case of streptococcal pharyngitis in any of the schools, during the last 4 to

5 surveys (Fig. 7.3), again emphasissing the impact of the programme.

4) As a result of the primary prophylaxis instituted and concurrent with the control of streptococcal pharyngitis, RF incidence also was controled and no new case RF or RHD occurred during the last year of the study.

It is concluded that, control of clinical and subclinical cases of streptococcal pharyngitis and complete prevention of RF and the morbidity and mortality of chronic RHD and its recurrences is possible in India, if a concerted attempt is made by a team of epidemiologists, microbiologists and cardiologists. Health education is of primary importance and providing free treatment for some years to come will be a necessity. The success of the present demonstration was made possible mainly because of the team work and the generous grant from the Netherlands Goverment.

TABLE 7.1

AGE-SEX DISTRIBUTION OF 2577 CHILDREN REGISTERED FOR THE SURVEILLANCE STUDY
(1st SURVEY)

| VILLAGE/ SCHOOL | MALE AGE IN YEARS 4-5 | 6-10 | 11-15 | TOTAL | FEMALE AGE IN YEARS 4-5 | 6-10 | 11-15 | TOTAL | GRAND TOTAL |
|---|---|---|---|---|---|---|---|---|---|
| V02/RS05 (HS) | 22 | 208 | 129 | 359 | 22 | 146 | 79 | 247 | 606 |
| V04/RS02 | 6 | 40 | 29 | 75 | 4 | 25 | 17 | 46 | 121 |
| RS09 | 4 | 38 | 5 | 47 | 11 | 37 | 5 | 53 | 100 |
| V07/RS01 | 14 | 59 | 1 | 74 | 27 | 53 | - | 80 | 154 |
| RS03 (HS) | - | 32 | 261 | 293 | - | 17 | 105 | 122 | 415 |
| RS08 | 9 | 50 | 9 | 68 | 13 | 52 | 2 | 67 | 135 |
| V08/RS11 | 4 | 44 | 3 | 51 | 15 | 46 | 2 | 63 | 114 |
| V09/RS10 | 11 | 36 | 1 | 48 | 2 | 33 | - | 35 | 83 |
| V10/RS07 | 16 | 56 | 6 | 78 | 11 | 25 | 3 | 39 | 117 |
| V11/RS06 | 3 | 35 | - | 38 | 9 | 39 | - | 48 | 86 |
| V21/RS12 | 11 | 75 | 33 | 119 | 4 | 40 | 16 | 60 | 179 |
| RS13 | 1 | 5 | - | 6 | 4 | 10 | - | 14 | 20 |
| RS14 | 14 | 62 | 2 | 78 | 9 | 37 | 1 | 47 | 125 |
| V23/RS16 | 12 | 49 | 1 | 62 | 5 | 29 | - | 34 | 96 |
| V25/RS15 | 12 | 108 | 25 | 145 | 3 | 72 | 6 | 81 | 226 |
| TOTAL | 139 | 897 | 505 | 1541 | 139 | 661 | 236 | 1036 | 2577 |
| PERCENT | 5.4 | 34.8 | 19.6 | 59.8 | 5.4 | 25.6 | 9.2 | 40.2 | 100.0 |

TABLE 7.2

ATTENDANCE AND ABSENTISM OF PUPILS IN 15 SCHOOLS DURING 9 SURVEYS 1975-1978

| SURVEY NO. | NO. ATTENDED | PERCENT ATTENDANCE | PERCENT ABSENTISM |
|---|---|---|---|
| I | 2577 | 100 | - |
| II | 2382 | 92 | 8 |
| III | 2396 | 93 | 7 |
| IV | 2520 | 98 | 2 |
| V | 2430 | 94 | 6 |
| VI | 2688 | 100 | - |
| VII | 2600 | 100 | - |
| VIII | 2271 | 88 | 12 |
| IX | 1703* | 88 | 12 |

* IXth survey was not done in schools RS12 to RS16, as holidays interfered.

TABLE 7.3

RECOVERY OF BHS AND ABHS FROM THROAT OF ALL CHILDREN WITH PHARYNGITIS BELONGING TO 15 SCHOOLS IN 9 SURVEYS

| SURVEY NO. | NO. PUPILS | BHS % | ABHS % |
|---|---|---|---|
| I | 2577 | 3.1 | 0.9 |
| II | 2382 | 1.5 | 0.5 |
| III | 2396 | 0.6 | 0.1 |
| IV | 2520 | 1.0 | 0.6 |
| V | 2430 | 0.1 | 0.08 |
| VI | 2688 | 0.1 | - |
| VII | 2600 | 0.03 | - |
| VIII | 2271 | 0.2 | 0.04 |
| IX* | 1703 | - | - |

* IXth survey not done in RS12 to RS16, as holidays interfered.

TABLE 7.4

RECOVERY OF BHS AND ABHS FROM THROAT CULTURES OF THOSE WITH PHARYNGITIS AMONG THE 923 CHILDREN WHO ATTENDED ALL SURVEYS IN THE 15 SCHOOL

| SURVEY NO. | BHS % | ABHS % |
|---|---|---|
| I | 3.0 | 0.5 |
| II | 2.4 | 0.9 |
| III | 0.4 | - |
| IV | 0.7 | 0.1 |
| V | 0.1 | - |
| VI | 0.1 | - |
| VII | - | - |
| VIII | 0.1 | - |
| IX* | - | - |

* IX Survey not done in RS12 to RS16 only 689 children attended in the other 10 schools during IX survey.

TABLE 7.5

RESULTS OF THREE STREPTOCOCCAL ANTIBODY TESTS DONE IN 23 CHILDREN WITH PHARYNGITIS

| | % SIGNIFICANT* TITRE |
|---|---|
| ASO | 30.4 |
| AH | 52.2 |
| ADNB | 91.3 |
| ANY ONE ANTIBODY | 91.3 |

| | | |
|---|---|---|
| * SIGNIFICANT TITRES | ASO | $\geq$ 340 Units |
| | AH | $\geq$ 1024 Units |
| | ADNB | $\geq$ 800 Units |

TABLE 7.4.B

T-PATTERNS OF 47 ABHS ISOLATED FROM PHARYNGITIS IN CHILDREN OF 15 SCHOOLS DURING ALL NINE SURVEYS

| T-TYPES | I | II | III | IV | V | VI | VII | VIII | IX | TOTOL | % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 3 (13.6) | | | | | | | | | 3 | 6.4 |
| 3/13/B 3264 | 5 (22.7) | 3 (21.4) | | 1 (16.7) | | | | | | 9 | 19.1 |
| 2 | | 1 (7.1) | | | | | | | | 1 | 2.1 |
| 4/28 | 5 (22.7) | 2 (14.3) | | 2 (13.3) | | | | | | 9 | 19.1 |
| 5/11/12/27/44 | 3 (13.6) | 4 (28.6) | 2 (100) | | | | | | | 9 | 19.1 |
| 8/25/IMP 19 | 4 (18.2) | | | 2 (100) | | | | | | 6 | 12.8 |
| 14 | 2 (9.1) | 4 (28.6) | | 3 (50.0) | | | | | | 9 | 19.1 |
| 9 | | | | | | 1 (100) | | | | 1 | 2.1 |
| 22, 23 | | | | | | | | | | | |
| TOTAL POSITIVE | 22 | 14 | 2 | 6 | 2 | | | 1 | | 47 | |
| UNTYPABLE | 1 | | | | | | | | | 1 | 2.1 |
| TOTAL STRAINS | 23 | 14 | 2 | 6 | 2 | | | 1 | | 48 | |

TABLE 7.6

RESULTS OF MULTIPLE STREPTOCOCCAL ANTIBODY TESTS DONE IN VARYING NUMBER OF CHILDREN WITH PHARYNGITIS

| | NO. DONE | NO. POS. | PERCENT SIGNIFICANT* TITRE |
|---|---|---|---|
| ASO | 300 | 105 | 35.0 |
| AH | 308 | 123 | 39.9 |
| ADNB | 58 | 50 | 86.2 |

* Legend as in 7.5

TABLE 7.7

PRIMARY PROPYLAXIS IN STREPTOCOCCAL PHARYNGITIS OF CHILDREN IN 15 SCHOOLS

| SURVEY NO. | PERCENT PROPHYLAXIS DONE |
|---|---|
| I | 44.0 |
| II | 60.4 |
| III | 60.0 |
| IV | 100.0 |
| V | 100.0 |
| VI | 100.0 |
| VII | 50.0 |
| VIII | 100.0 |
| IX | 100.0 |

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# CHAPTER 8

## STREPTOCOCCAL PYODERMA IN RURAL SCHOOL CHILDREN IN SOUTHERN INDIA AND THE IMPACT OF PROPHYLAXIS

It is believed that the second most common infection with beta-hemolytic streptococci (BHS) in man is that of the skin. Streptococcal workers all over the world have been so preoccupied with streptococcal infection of the pharynx (referred to here-after as Pharyngitis), that, very little attention has been given to streptococcal infection of the skin[1]. However, there has been an increasing interest in skin infections during recent years. The enthusiasm of streptococcal workers for pyoderma during the past two decades had resulted in a better understanding of the agent and the disease.

Any streptococcal infection of the skin may be defined as pyoderma[2]. Pyoderma may be manifested in a veriety of clinical forms such as vesicular, pustular, crusted and may present as single or coalescent lesions depending on the stage of the lesion causative agent or agents concerned. Streptococcal pyoderma may be primary or secondary; usually the primary streptococcal pyoderma appears as a papular or transiently vesicular, going on to a pustular lesion which later presents as a discrete, crusted, non scarring lesion[1]. Such a crusted lesion is referred to as an impetigo. Some primary impetigo caused by Staphylococcus aureus may be secondarily infected by streptococci. Bullous impetigo are invariably

staphylococcal in etiology.

Streptococcal pyoderma has a small diffuse erythema surrounding the papular or pustular lesion. True vesicles may often be missed. Pain is rare and regional lymphadenitis is not common unlike streptococcal pharyngitis. Streptococcal infections of wounds and burns are also not rare. The pure form of erysipelas, rare in tropical countries and also in temperate climate in recent years are not included under pyoderma.

Wannamaker[1] in a masterly review, has brought out the differences between streptococcal infections of the throat and of the skin. Superficial streptococcal infection of the skin often pursue a chronic course with individual lesions lasting for one week or longer and deeper cutaneous lesions lasting for weeks or months.

The more common association of BHS in early lesions of pyoderma suggest a more significant role for streptococci, than the staphylococci, in the initiation of impetigo. Intensive study and sequential culturing has further confirmed the important role for BHS in the impetigo scene. Streptococci of the same serotype as found in impetigo are also harboured by children in the nose and throat[1,2,3].

The concern of clinicians, microbiologists, and epidemiologists in streptococcal pyoderma in recent years is mainly because of the sequelae it produces, namely, post streptococcal glomerulonephritis (PSGN). The association of streptococcal pyoderma with PSGN has been recognised for

many years [2,3]. The association of skin infection with acute glomerulonephritis has been found more often in tropical and subtropical climates [4-8]. Such an association was observed in patients attending outpatient clinics by Koshi et al [4,9]. The higher prevalence of streptococcal pyoderma over streptococcal pharyngitis was noticed in the pilot study [10] on surveillance of streptococcal infection among children of rural and urban schools. The result of the pilot study revealed the need for a detailed surveillance of streptococcal infection. Such a surveillance of streptococcal pyoderma was undertaken among children of 15 rural schools in Southern India with the following objectives:-

1. To find out the magnitude of the problem of streptococcal pyoderma in school age children in rural Southern India.

2. To study the bacteriology and serology of streptococcal pyoderma in children.

3. To deleniate the epidemiological factors involved in the higher prevalence (as noted in the pilot study [10]) of streptococcal pyoderma over streptococcal pharyngitis.

4. To institute control programmes and prophylaxis in streptococcal pyoderma and to note its impact on the disease in the community and if possible, on the incidence of PSGN.

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## SUBJECTS AND METHODS

The subjects included and the methods employed have been given in detail in Chapter 2 and was the same as those included for the pharyngitis surveillance (Chapter 7). The data on the children included is given in Table 2.2.

The age-sex distribution of children included in this study was the same as was detailed in Chapter 7 (Table 7.1). The number seen in each survey and the absentism etc. are entered in Table 7.2.

Each school was visited and children surveyed at least thrice in the year to correspond to the three different seasons of the year and to adjust to the working days. The member card (Appendix I-B) of each child was filled in. When pyoderma was suspected in any child, the pyoderma card was filled in for all clinical symptoms & signs (Appendix I-D). Further, details of similar history in siblings, parents and neighbours were also recorded. Attempt was made to follow up the absentees repeatedly.

Whenever a child was suspected to have pyoderma, swabs were collected from skin lesions as described in Chapter 2 & whenever possible, from throat and nose also. The multiple transportation technique carried out in the earlier part of the study (Refer Chapter 3, Appendix III) proved that the filter paper (F.P) technique was as good as the inoculation of skin swabs on blood agar in the field. Hence, for simplicity, convenience and for efficacy, the F.P. technique was followed for subsequent surveys. Moreover for throat

swabs, the F.P. technique have been found superior.

The F.P. was cultured on 5% sheep blood agar and incubated at 35°C ± 1°C. Further steps followed are detailed in Chapter 2 & 3. For the culture of swabs from normal skin, crystal violet blood agar (CVBA) plates (Appendix II) were used as it was found to be superior to BA; it could also selectively inhibit staphylococci and the contaminants from the skin, leaving streptococci untouched. Other procedures followed were the same as for throat cultures. Grouping and typing of BHS were done according to conventional procedures (Appendix III). Staphylococci isolated which had minimal role were also characterised, though the report is not included here.

Whenever a child did not object, about 5 ml of blood was collected for streptococcal antibody titration. The antibody tests carried out were antistreptolysin O (ASO), antihyaluronidase (AH) & anti-deoxyribonuclease B (ADNB).

Pupils who were proved to have streptococcal pyoderma either by BHS isolation and/or significant antibody titres were given treatment (prophylaxis) with one injection of Benzhathine Benzyl Penicillin 600,000 units, if below 5 years and 1,200,000 units, if above 5 years.

## RESULTS

Code Rs 01 to Rs 16 given to the schools was followed for all studies. For the data on the age-sex of the children included in the study, represented school-wise, refer Table 7.1. Maximum number of children (60.4%) were in

the 6 to 10 age group, 28.8% in the 11-15 and 10.8% in the 4-5 age-group. The male to female ratio was 3:2. The attendance of pupils was kept up between 88 to 100% and absentism varied from nil to 12% in different surveys. New admissions made up for absentism and dropouts during the various surveys (Refer Table 7.2). In total, only 923 (35.5%) of the originally registered pupils, attended all the 9 surveys conducted, throughout the four-year study.

The isolation of BHS and ABHS from skin lesions were easy and contamination was not a problem. Data on clinical pyoderma, BHS and ABHS isolation from children with pyoderma, school-wise, during the first survey is given in Table 8.1. The prevalence percent of clinical pyoderma from all schools was 12.8, BHS isolation 9.4 and ABHS 7.7 (Table 8.1 & 8.3). The BHS and ABHS Isolation from 2577 children arranged according to the frequency of isolation from each school is represented in the bar diagram in Fig.8.1.

The isolation of BHS and ABHS was higher in males than in females (Table 8.2). The maximum isolation and overall isolation in each sex was at its highest (8.6%) in the 6 to 10 year age group, 6.9% in the 11 to 15 year age group and minimum (4.6%) in the 4 to 5 year age group.

The survey-wise point prevalence of BHS and ABHS in pyoderma is given in Table 8.3. Though there was no statistically significant difference in the point prevalence of ABHS pyoderma for each of the surveys, its incidence

varied from 5.9 to 9.8% with a mean of 7.3%.

Majority of the suspected pyoderma yielded BHS & ABHS; the recovery of BHS varied from 58 to 82.5% with a mean of 72.3% and ABHS varied from 46.9% to 74.5% with a mean of 61.6% (Table 8.4).

The recovery of BHS and ABHS from pyoderma among 923 children seen in all surveys is given in Table 8.5. The percent recovery of BHS varied from 6.6% to 12.4% with a mean of 9.2% of the total 923 children surveyed, while that of ABHS varied from 5.7% to 10.8% with a mean of 7.9%. The latter findings were more or less similar to the results in the overall group (Table 8.5 vs Table 8.3).

The school-wise and survey-wise details of ABHS recovery from pyoderma in the different surveys is given in Table 8.6 & Fig. 8.2 which show the percent recovery of BHS and ABHS in each of the 15 schools, survey-wise. It is very evident that the incidence of pyoderma was irregular in each school and in each survey. There was no particular pattern either school-wise or survey-wise. The highest prevalence of BHS and ABHS seen in the first survey of RS11 was repeated in the 5th and 6th surveys also, in RS11 as well as in RS06 (Fig.8.2). The point prevalence was higher during 2nd survey in school RS08 & RS10. In RS02 higher point prevalence was seen in the 5th, 7th, 8th and 9th surveys.

Details on BHS and ABHS from 923 children who attended all 9 surveys are given in Table 8.7 and Fig.8.3. Further details on antigenic groups encountered in each survey are

also represented. Large majority of pyoderma were caused by ABHS and accounted for 61.6% of all pyoderma (Table 8.4) and 69.3% of pyoderma of 923 children seen during all 9 surveys (Table 8.7). Group B streptococci were rare, while Group G were responsible for 6.7% and Group C for 3.9% of pyoderma in this population of 923 during the whole survey period (Table 8.7).

Multiple serological tests were very revealing in pyoderma as shown in 353 cases with BHS isolation & 220 cases with ABHS isolation (Fig.8.4 & Fig.6.2). With ABHS or with BHS the results of antibody tests were similar (Table 8.8). ASO was least rewarding in pyoderma, AH next best and ADNB gave significant titres in 88 to 90% of cases (Fig.8.5). Thus the value of doing multiple antibody tests were very evident (Table 8.8 and Fig. 8.5). Results of 3 antibody studies done on large number of children with clinical pyoderma, irrespective of BHS and ABHS recovery, were also comparable (Table 8.9).

Large majority of ABHS isolated were T-typable (98.8%) in our hands. The T type patterns were different from findings elsewhere (Table 8.10). The overall results on T types of 632 ABHS from 640 pyoderma lesions according to the frequency of their isolation is represented in Fig. 8.6. The commonest T type in all schools except RS10 & RS11, belonged to the T complex 3/13/B3264 followed closely by 4/28 & 5/11/12/27/44, while 8/25/Imp 19 complex was 6th in the order of frequency. T14 & T9 were the commonest single

T types, followed by 6, 1 & 2. T type distribution of ABHS isolated from each school-wise, is represented in Fig.8.7.

Very striking was the fact that different serotypes came up in different surveys and in different schools, well brought out in a scattergram survey-wise for two schools RS05 & RS03 (Fig. 8.8).

The pyoderma lesions had higher predilection for lower limbs (55.6%) being exposed to external injury and insect bites (Table 8.11). Next common was lower limbs with another site (10.1%), or multiple sites (11.8%), upper limbs in 10.7% and head including scalp, ear, etc. in 2.8% only.

Compliance to treatment in different schools varied from 50% to 100% of children with pyoderma (Table 8.12).

## DISCUSSION

The role of beta-hemolytic streptococci (BHS) in the causation of skin infection has been high lighted much in the past [5,6,7]. Koshi et al [4] in 1967 had reported a high rate of isolation of BHS from pyogenic infection of the skin of children attending an out patient clinic in Vellore, S. India. Acute glomerulonephritis following repeated attacks of streptococcal skin infection in a nine year-old girl was also reported at that time. Dillon et al [2,3] has written much about Group A streptococci (ABHS) causing pyoderma which preceeds acute glomerulonephritis. The major role of ABHS of pyoderma in causing post streptococcal glomerulonephritis (PSGN) in children of the tropics is now very well recognised. Though PSGN can follow streptococcal infection of the throat or skin [1], the primary cause of PSGN in the tropics, unlike that in temperate climates, have mainly been following skin infections. Large outbreaks of PSGN that have been reported from many hot and humid climates have resulted from outbreaks of scabies and subsequent streptococcal skin lesions [8]. Thus, as in rheumatic fever control and prevention, a regular surveillance programme and primary prophylaxis, will probably be contributory to the prevention of PSGN following streptococcal pyoderma in the tropics. However, due to the lesser seriousness of PSGN and almost complete cure of nephritis in childhood, primary prevention of PSGN is not

often practiced, unlike rheumatic fever (RF) and rheumatic heart disease (RHD).

In the present study, among rural school children, the major role of ABHS in causing pyoderma, in the 5-15 year age-group, was confirmed beyond doubt. The prevalence of streptococcal skin infection among the 2577 school children from 15 schools during the first survey was as high as 9.4%, with ABHS being responsible for 7.7% of skin infection; the rates varied from 4.1% to 12.8% in different schools (Table 8.1). Streptococcal pyoderma was higher in male children and the highest susceptible age group among both sexes was, as expected, the 6-10 year age-group (Table 8.2).

The better technique, we followed, for transportation of clinical materials from the field, the better combination of media & techniques used and the multiple improved grouping techniques carried out, were all responsible for the maximal recovery & identification of BHS & different antigenic groups. Thus, in different surveys we were able to isolate 72.3% of BHS from skin lesions while ABHS isolation varied from 55.5 to 74.5% with a mean of 61.6% of clinical pyodermas (Table 8.3).

Despite prompt primary prophylaxis of all streptococcal pyoderma, proved by culture and/or antibody rise, the incidence rate of the disease did not show any decrease. The point prevalence in each survey varied from 5.9% to 9.8% with a mean of 7.3% in all the surveys together (Table 8.4). An almost similar incidence of 5.7% to 10.8% with a

mean of 7.9% of ABHS pyoderma was observed in the 923 children who were followed up regularly in all the 9 surveys (Table 8.5). Thus, this study not only showed a high prevalence rate, but also a high endemicity of streptococcal pyoderma in this community. This is in marked contrast to our results of pharyngitis in the same population. As given in Chapter 7, BHS pharyngitis varied from 0.1% to 3.1% and ABHS pharyngitis varied from 0.04% to 0.9% in the different surveys (Refer Chaper 7, Table 7.3 and Fig. 7.4).

A multitude of epidemiological factors might have been responsible for the perpetuation and endemicity of streptococcal pyoderma in these school children. The low socio economic standard, unhygienic conditions at home, overcrowding at home & school, resulting in close contact, scabies, mosquitoe bites, louse bites, abrasions & injury, all getting infected each time from the skin and environmment in the dusty, hot & humid climate, scarcity of water in summer months to keep the skin clean, use of common towel, clothes, soaps & combs, may all have contributed to the endemicity of this disease in school going children.

The high predilection of pyoderma lesions of the lower extremities was striking in the present study, as expected of all those who go about without foot wears. The rate was comparatively similar to pre-school goers & non-school goers (absentees) who were,for a time surveyed,at home (Chapter 9). The high variations in the prevalence rates could also be due to the new entries of children into the school during

each survey.

It is interesting to note that the highest prevalence in most of the schools was during the 7th and 8th surveys which corresponded to a period when we had unprecedented cyclonic storm in South India including Vellore. Probably the very hot & humid period just preceding the cyclonic storm might have been one of the factors reponsible for outbreaks of pyoderma. Moreover, in a certain village (V23) and corresponding school (RS16) there were epidemics of scabies affecting both children & adults and with multiple members of families having scabies & pyoderma. Some of these children might have contributed for perpetuating pyoderma in schools and community. Our attempts at primary prophylaxis of all proved streptococcal infection along with health education and practical demonstration succeeded in preventing epidemics of PSGN in V23 & RS16.

Group ABHS was undoubtedly reponsible for a large majority of streptococcal pyoderma we encountered in all the schools at all surveys and contributed to 61.6% clinical pyoderma (Table 8.4). However, the role of non-group ABHS cannot be ignored. Group B streptococci were noted for their absence except in 2 cases; Group G, on the other hand, was isolated in moderate to heavy growth (Table 8.7) and in pure culture in 6.7% of all pyodermas, while Group C was associated with 3.9% of pyodermas. Thus, the role of Groups G & C in pyodermas among these children can no longer be ignored. In fact, as early as 1967, Koshi[4,9] had reported

on the high prevalence of Groups G & C in pyodermas of children attending outpatient clinic. Role of Groups G & C in pyoderma have now been placed on firmer grounds in this long term surveillance in rural South Indian children. Similar findings have also been reported in other tropical & subtropical countries[10,11,12].

The role of antibody studies in the confirmation of streptococcal pyoiderma have been widely discussed in the past[13,14]. As highlighted by others[15], the present study also confirmed the limitations of ASO in the serodiagnosis of pyoderma (Table 8.8); AH was found to be useful adjunct. However, ADNB was found to be the most sensitive test for serological documentation of pyoderma. In fact, this latter test was positive in, more than double the number of cases as against ASO (Table 8.8) in our study. Furthermore, even the addition of a second test such as AH, could detect only 3% more than what ADNB could detect (Table 8.8). This confirms the need for doing ADNB and its superiority in the confirmation of streptococcal pyoderma, which has also been reported elsewhere[14,15]. However, this should not underscore the value of doing multiple antibody tests which, in our series, could document as much as 95% of all Group A streptococcal pyoderma (Table 8.8) and 94% of all clinically suspected pyoderma (Fig. 8.5).

The nephritogenic capacity of certain types of Group A streptococci is well established[16,17]. By the precipitin technique, Markowitz[5] *et al* were able to type only four out

of 53 Group A strains from 303 children with impetigo, thougth by agglutination they were able to type 21 strains. Parker et al[18] and Barrow[19] showed that group A streptococci from impetiginous lesions fell into one of three agglutination patterns. Moody et al[20] reported that in 1963 they could type, by M-precipitation, only eight out of the 94 strains isolated from throat and skin lesions of children with impetigo. The feasibility and usefulness of T-agglutination for serotyping of streptococci have been further confirmed by Moody et al[20] in their extensive comparative study of M-precipitation and T-agglutination.

The distribution of various T types involved in pyoderma lesions showed recognized "impetigo strains", though the percent frequency was different. 3/13/B3264, a common T type found associated with skin sesions, was the most predominant, followed by 4/28 and 5/11/12/27/44. T complex 8/25/Imp 19, a group which is reported to be very frequently associated with pyoderma[1,2] in many studies accounted for only 8.4% of all ABHS in this study (Table 8.10). However, in general, the T typing patterns were essentially similar to many studies[18,20,21] emphasising similarity in epidemiological trends in pyoderma.

An important observation during the study was the changing pattern of the T-type involved in pyoderma during different surveys (Table 8.10 & Fig. 8.8). For example, in RS05, T type 8/25 Imp 19 were seen predominantly during 1st survey and then gradually were replaced by other types. On

the other hand, 5/11/12/27/44 and 3/13/B3264 were seen predominantly during 1st, 4 th, 5th, 7th and 9th surveys. It is probable that this reflects on the prevalence of some M types in the community during certain periods and others, at other times.

Compliance in primary prophylaxis for streptococcal pyoderma is important for the prevention of its sequelae. As seen in Table 8.12, this increased from 50% during first survey to 100% during 5th survey. To our satisfaction, a compliance of 80% to 99% was achieved at different surveys. Adequate health education and close understanding of the rural community by the field workers/health educationists were found to be mandatory for such a high compliance.

Thus, in conclusion, the present study of streptococcal pyoderma among rural south Indian children not only showed a higher rate of prevalence of pyoderma than of pharyngitis, but also confirmed the high endemicity of pyoderma. A multitude of environmental factors including hot and humid climate, poor socioeconomic & hygienic conditions, as well as overcrowding were contributory to the endemicity. The study also confirmed that a high prophylactic coverage, adequate health education and a close surveillance, are all necessary, for the prevention of post streptococcal glomerulonephritis.

TABLE 8.1

PREVALENCE OF CLINICAL PYODERMA, BHS AND ABHS FROM CHILDREN WITH PYODERMA AMONG 2577 CHILDREN SEEN DURING I SURVEY

| SCHOOL | NO. SEEN | CLINICAL PYO. NO. | % | BHS NO. | % | ABHS NO. | % |
|---|---|---|---|---|---|---|---|
| V02/RS05 | 606 | 95 | 15.7 | 76 | 12.5 | 53 | 8.7 |
| V04/RS02 | 121 | 15 | 12.4 | 12 | 9.9 | 11 | 9.1 |
| RS09 | 100 | 12 | 12.0 | 10 | 10.0 | 9 | 9.0 |
| V07/RS01 | 154 | 21 | 13.6 | 14 | 9.1 | 12 | 7.8 |
| RS03 | 415 | 36 | 8.7 | 19 | 4.6 | 17 | 4.1 |
| RS08 | 135 | 18 | 13.3 | 14 | 10.4 | 10 | 7.4 |
| V08/RS11 | 114 | 18 | 15.8 | 15 | 13.2 | 14 | 12.3 |
| V09/RS10 | 83 | 10 | 12.0 | 9 | 10.8 | 9 | 10.8 |
| V10/RS07 | 117 | 17 | 14.5 | 12 | 10.3 | 8 | 6.8 |
| V11/RS06 | 86 | 18 | 20.9 | 15 | 17.4 | 11 | 12.8 |
| V21/RS12 | 179 | 19 | 9.5 | 12 | 6.7 | 11 | 6.1 |
| RS13 | 20 | 1 | 5.0 | 1 | 5.0 | 1 | 5.0 |
| RS14 | 125 | 14 | 11.2 | 8 | 6.4 | 8 | 6.4 |
| V23/RS16 | 96 | 12 | 12.5 | 8 | 8.3 | 8 | 8.3 |
| V25/RS15 | 226 | 25 | 11.1 | 17 | 7.5 | 16 | 7.1 |
| TOTAL | 2557 | 331 | | 242 | | 198 | |
| PERCENT | | | 12.8 | | 9.4 | | 7.7 |

TABLE 8.2

AGE SEX DISTRIBUTION OF CHILDREN (2577) AND ABHS ISOLATION FROM CHILDREN WITH PYODERMA SEEN IN 15 SCHOOLS

| SEX | NO REGD. | BHS NO. | RECOVERY % | AGE-WISE RECOVERY OF ABHS 4-5 YRS | 6-10 YRS | 11-15 YRS |
|---|---|---|---|---|---|---|
| MALE | 1541 | 167 | 10.8 | 6.5 | 9.9 | 7.5 |
| FEMALE | 1036 | 75 | 7.2 | 2.9 | 6.8 | 5.5 |
| TOTAL | 2577 | 242 | 9.4 | 4.6 | 8.6 | 6.9 |

TABLE 8.3

PRECENT ISOLATION OF BHS AND ABHS FROM SUSPECTED PYODERMA FROM ALL SCHOOLS DURING 9 SURVEYS

| SURVEY | CLINICAL PYODERMA | BHS NO. | BHS % | ABHS NO. | ABHS % |
|---|---|---|---|---|---|
| I | 331 | 242 | 73.1 | 198 | 59.8 |
| II | 211 | 150 | 71.1 | 140 | 66.4 |
| III | 215 | 161 | 74.9 | 148 | 68.8 |
| IV | 212 | 175 | 82.5 | 158 | 74.5 |
| V | 274 | 198 | 72.3 | 152 | 55.5 |
| VI | 347 | 202 | 58.2 | 163 | 46.9 |
| VII | 382 | 294 | 76.9 | 255 | 66.8 |
| VIII | 326 | 239 | 73.3 | 214 | 65.6 |
| IX | 232 | 167 | 72.0 | 131 | 56.5 |
| TOTAL | 2530 | 1828 | 72.3 | 1559 | 61.6 |

TABLE 8.4

CLINICAL PYODERMA, BHS AND ABHS RECOVERY FROM TOTAL CHILDREN SEEN BELONGING TO 15 SCHOOLS DURING 9 SURVEYS

| SURVEY NO. | NO. PUPILS | CLINICAL PYODERMA | | BHS | | ABHS | |
|---|---|---|---|---|---|---|---|
| | | NO. | % | NO. | % | NO. | % |
| I | 2577 | 331 | 12.8 | 242 | 9.4 | 198 | 7.7 |
| II | 2382 | 211 | 8.9 | 150 | 6.3 | 140 | 5.9 |
| III | 2396 | 215 | 9.0 | 161 | 6.7 | 148 | 6.2 |
| IV | 2520 | 212 | 8.4 | 175 | 6.9 | 158 | 6.3 |
| V | 2340 | 274 | 11.7 | 198 | 8.5 | 152 | 6.5 |
| VI | 2688 | 347 | 12.9 | 202 | 7.5 | 163 | 6.1 |
| VII | 2600 | 382 | 14.7 | 294 | 11.3 | 255 | 9.8 |
| VIII | 2271 | 326 | 14.4 | 239 | 10.5 | 214 | 9.4 |
| IX | 1703 | 232 | 13.6 | 167 | 9.8 | 131 | 7.7 |
| TOTAL | 21477 | 2530 | 11.8 | 1828 | 8.5 | 1559 | 7.3 |

TABLE 8.5

CLINICAL PYODERMA AND RECOVERY

OF BHS AND ABHS FROM 923 CHILDREN SEEN IN ALL 9 SURVEYS

| Survey | Clinical Pyoderma | | B H S | | A B H S | |
|---|---|---|---|---|---|---|
| No. | No. | % | No. | % | No. | % |
| I | 139 | 15.1 | 100 | 10.8 | 79 | 8.6 |
| II | 84 | 9.1 | 63 | 6.8 | 60 | 6.5 |
| III | 90 | 9.8 | 66 | 7.2 | 63 | 6.8 |
| IV | 77 | 8.3 | 61 | 6.6 | 56 | 6.1 |
| V | 115 | 12.5 | 92 | 10.0 | 74 | 8.0 |
| VI | 108 | 11.7 | 66 | 7.2 | 53 | 5.7 |
| VII | 147 | 15.9 | 113 | 12.4 | 100 | 10.8 |
| VIII | 139 | 15.1 | 100 | 10.8 | 96 | 10.4 |
| IX | 102 | 14.8 | 78 | 11.3 | 59 | 8.6 |
| Total (8073 children) | 1001 | 12.4 | 739 | 9.12 | 640 | 7.9 |

* Number seen in IXth survey = 689

TABLE 8.6

POINT PREVALENCE (INCIDENCE) OF ABHS IN PYODERMA AMONG 923 CHILDREN SEEN SCHOOL-WISE DURING 9 SURVEYS

| SCHOOLS | NO. SEEN IN ALL, SURVEYS | PERCENT ABHS ISOLATION IN SURVEY I ABHS | II ABHS | III ABHS | IV ABHS | V ABHS | VI ABHS | VII ABHS | VIII ABHS | IX ABHS |
|---|---|---|---|---|---|---|---|---|---|---|
| RS11 | 29 | 17.2 | 6.9 | 3.4 | 3.4 | 17.2 | 17.2 | 10.3 | 10.3 | 6.9 |
| RS06 | 18 | 11.1 | 5.6 | - | 5.6 | 16.7 | 22.2 | - | 11.1 | 5.6 |
| RS05 | 291 | 10.0 | 7.2 | 5.8 | 5.8 | 8.2 | 2.1 | 14.1 | 10.3 | 11.7 |
| RS09 | 38 | 10.5 | 7.9 | 5.3 | - | 2.6 | 2.6 | - | 5.2 | - |
| RS02 | 32 | 9.4 | 3.1 | - | 9.4 | 15.7 | 9.4 | 18.8 | 34.4 | 21.9 |
| RS08 | 57 | 10.5 | 15.8 | 8.8 | 8.8 | 7.0 | 1.8 | 1.8 | 5.3 | 5.3 |
| RS03 | 100 | 10.0 | 5.0 | 8.0 | 8.0 | 2.0 | 5.0 | 9.0 | 13.0 | 2.0 |
| RS10 | 19 | 10.5 | 15.7 | 15.8 | 10.5 | 10.5 | 5.3 | 21.0 | 10.5 | 10.5 |
| RS07 | 32 | 6.3 | 9.3 | 6.3 | 9.4 | 9.4 | 6.3 | 21.9 | 18.7 | 3.1 |
| RS12 | 64 | 6.3 | - | 7.8 | 3.1 | 3.1 | 7.8 | 9.4 | 1.6 | - |
| RS15 | 89 | 5.5 | 2.2 | 4.5 | 3.4 | 7.5 | 10.1 | 11.2 | 8.9 | - |
| RS01 | 73 | 6.9 | 4.1 | 16.4 | 8.2 | 9.6 | 4.1 | 8.2 | 12.3 | 9.6 |
| RS14 | 37 | 5.4 | 10.8 | - | 2.7 | 5.4 | 5.4 | 11.8 | 8.8 | - |
| RS16 | 40 | 2.5 | 7.5 | 10.0 | 10.0 | 17.5 | 15.0 | 7.5 | 7.5 | - |
| RS13 | 4 | - | - | - | - | - | - | - | - | - |

TABLE 8.7

ANTIGENIC GROUPS OF BHS ISOLATED FROM PYODERMA OF 923 CHILDREN SEEN DURING NINE SURVEYS

| SURVEY | I | II | III | IV | V | VI | VII | VIII | IX | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|
| NO. OF CLINICAL PYODERMA | 139 | 84 | 90 | 77 | 115 | 108 | 147 | 139 | 102 | 1001 |
| NO. OF BHS | 100 | 63 | 66 | 61 | 92 | 66 | 113 | 100 | 78 | 739 |
| % OF SUSPECTS | 71.9 | 75.0 | 73.3 | 79.2 | 80.0 | 61.1 | 76.9 | 71.9 | 76.5 | 73.8 |
| % OF TOTAL (923) | 10.8 | 6.8 | 7.2 | 6.6 | 10.0 | 7.2 | 12.4 | 10.8 | 11.3 | 80.0 |
| NO. OF ABHS | 79 | 60 | 63 | 56 | 74 | 53 | 100 | 96 | 59 | 640 |
| % OF BHS | 79.0 | 95.2 | 95.5 | 91.8 | 80.4 | 80.3 | 88.5 | 96.0 | 75.6 | 86.6 |
| % OF TOTAL (923) | 8.6 | 6.5 | 6.8 | 6.1 | 8.0 | 5.7 | 10.8 | 10.4 | 8.6 | 69.3 |
| B | - | - | - | - | - | - | 1 (0.1) | - | - | 1 (0.1) |
| C | 5 (0.5) | - | 3 (0.3) | 2 (0.2) | 5 (0.5) | 6 (0.7) | 5 (0.5) | 2 (0.2) | 8 (1.2) | 36 (3.9) |
| G | 16 (1.7) | 3 (0.3) | - | 3 (0.3) | 13 (1.4) | 7 (0.8) | 7 (0.8) | 2 (0.2) | 11 (1.6) | 62 (6.7) |

TABLE 8.8

COMPARISON OF THREE ANTIBODY TESTS IN CHILDREN WITH PYODERMA

| SEROLOGY | PERCENT PYODERMA WITH BHS (n = 353) | PERCENT PYODERMA WITH ABHS (n = 220) |
|---|---|---|
| ASO | 41 | 40 |
| AH | 65 | 66 |
| ADNB | 88 | 90 |
| ANY ONE Ab. | 94 | 95 |
| AH + ADNB | 94 | 93 |
| ASO + ADNB | 91 | 93 |
| ASO + AH | 75 | 76 |

TABLE 8.9

RESULTS OF THREE ANTIBODY TESTS DONE ON CHILDREN WITH PYODERMA

| SEROLOGY | NO. DONE | SIGNIFICANT TITRE | |
|---|---|---|---|
| | | NO. | % |
| ASO | 736 | 315 | 42.8 |
| AH | 740 | 423 | 57.2 |
| ADNB | 364 | 316 | 86.8 |

TABLE 8.10

T-PATTERNS OF 640 ABHS ISOLATED FROM PYODERMA IN 923 CHILDERN SEEN IN 15 SCHOOLS DURING ALL NINE SURVEYS

| T-TYPES | I | II | III | IV | V | VI | VII | VIII | IX | TOTAL | % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3/13/B3264 | 16<br>20.8 | 11<br>18.6 | 19<br>32.2 | 10<br>17.9 | 18<br>24.3 | 15<br>28.3 | 29<br>29.3 | 19<br>19.8 | 18<br>30.5 | 155 | 24.2 |
| 4/28 | 15<br>19.5 | 11<br>18.6 | 14<br>23.7 | 17<br>30.4 | 7 | 4 | 15<br>15.2 | 20<br>20.8 | 12<br>20.3 | 115 | 18.0 |
| 5/11/12/27/44 | 13<br>16.9 | 14<br>23.7 | 4 | 3 | 15<br>20.3 | 12<br>22.6 | 13<br>13.1 | 12<br>12.5 | 3 | 89 | 13.9 |
| 14 | 4 | 14<br>23.7 | 4 | 5 | 7<br>9.5 | 8<br>15.1 | 13<br>13.1 | 10<br>10.4 | 5<br>8.5 | 70 | 10.9 |
| 9 | 6 | 2 | 2 | 6 | 5 | 5 | 15<br>15.2 | 15<br>15.6 | 4 | 60 | 9.4 |
| 8/25/Imp 19 | 16<br>20.8 | 5 | 11<br>8.6 | 8 | 7<br>9.5 | 1 | 1 | 1 | 4 | 54 | 8.4 |
| 6 | 4 | - | 4 | 5 | 10<br>13.5 | 4 | 3 | 7 | 4 | 41 | 6.4 |
| 1 | 3 | - | 1 | 1 | 2 | 3 | 3 | 4 | 4 | 21 | 3.3 |
| 2 | - | - | - | 1 | 2 | 1 | 5 | 8<br>8.3 | 4 | 21 | 3.3 |
| 22,23 | - | 2 | - | - | 1 | - | 2 | - | 1 | 6 | 0.9 |
| TOTAL POSITIVE | 77 | 59 | 59 | 56 | 74 | 53 | 99 | 96 | 59 | 632 | 98.7 |
| NOT TYPABLE OR LOST | 2 | 1 | 4 | - | - | - | 1 | - | - | 8 | 1.3 |
| TOTAL NUMBER | 79 | 60 | 63 | 56 | 74 | 53 | 100 | 96 | 59 | 640 | 100.0 |

TABLE 8.11

DISTRIBUTION OF PYODERMA LESIONS SEEN IN SCHOOL CHILDREN DURING FIRST SURVEY (2577)

| SITE | BHS | | ABHS | |
|---|---|---|---|---|
| | NO. | % | NO. | % |
| LOWER LIMB | 123 | 57.7 | 99 | 55.6 |
| UPPER LIMB | 19 | 8.9 | 19 | 10.7 |
| HEAD | 7 | 3.3 | 5 | 2.8 |
| LESIONS AT 2 SITES | 40 | 18.8 | 34 | 19.1 |
| LESIONS >2 SITES | 24 | 11.2 | 21 | 11.8 |
| TOTAL | 213 | 100.0 | 178 | 100.0 |

TABLE 8.12

PRIMARY PROPHYLAXIS IN STREPTOCOCCAL PYODERMA, SURVEY-WISE

| SURVEY NUMBER | PERCENT PROPHYLAXIS IN PYODERMA |
|---|---|
| I | 50.0 |
| II | 60.9 |
| III | 67.6 |
| IV | 91.6 |
| V | 100.0 |
| VI | 98.9 |
| VII | 89.2 |
| VIII | 90.6 |
| IX | 79.5 |

PREVALENCE PERCENT OF STREPTOCOCCAL PYODERMA IN 2577 CHILDREN SEEN IN THE FIRST SURVEY

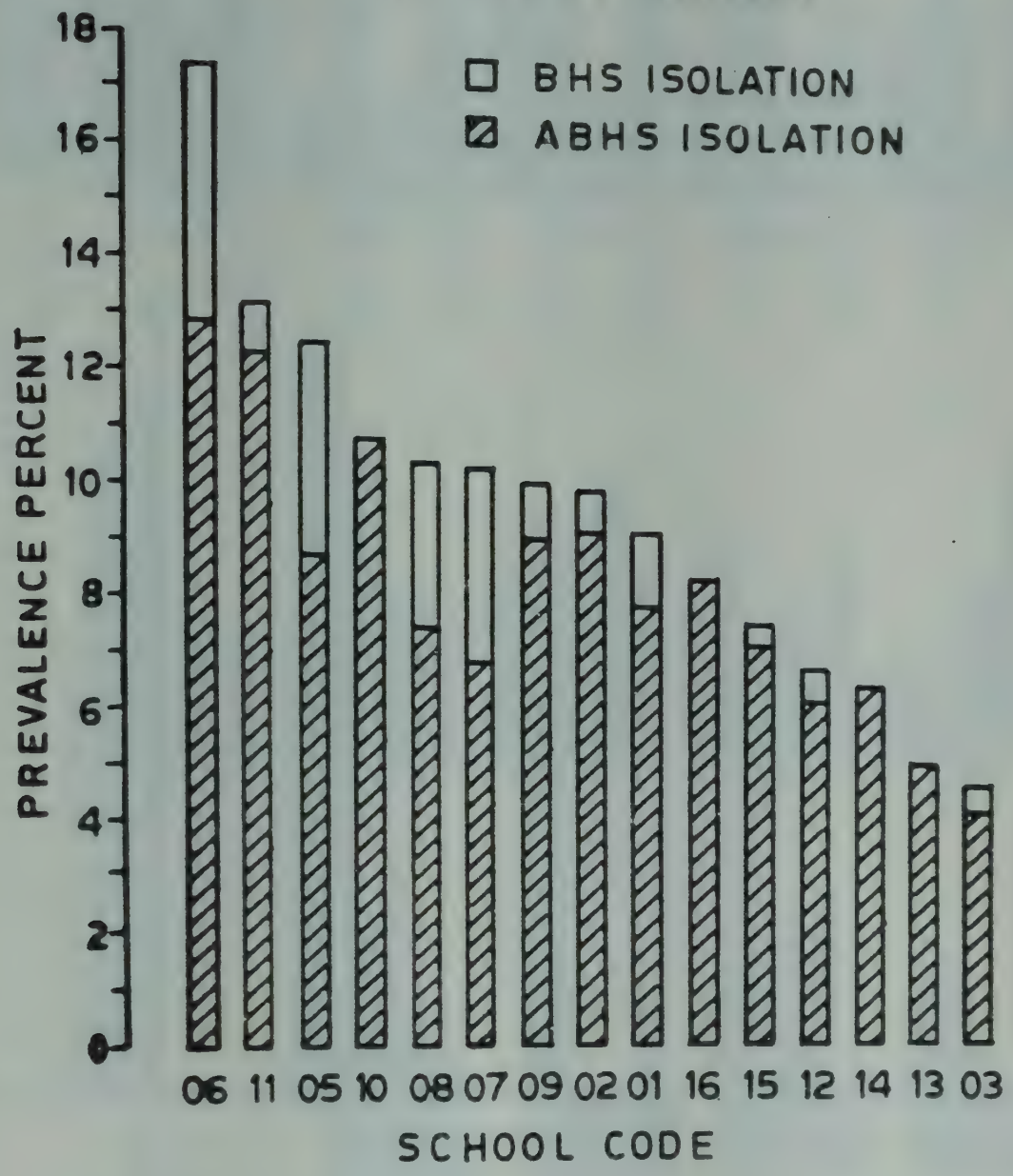



FIG. 8.1

FIG. 8.2 INCIDENCE PERCENT OF STREPTOCOCCAL PYODERMA IN 923 CHILDREN OF 15 SCHOOLS IN ALL 9 SURVEYS

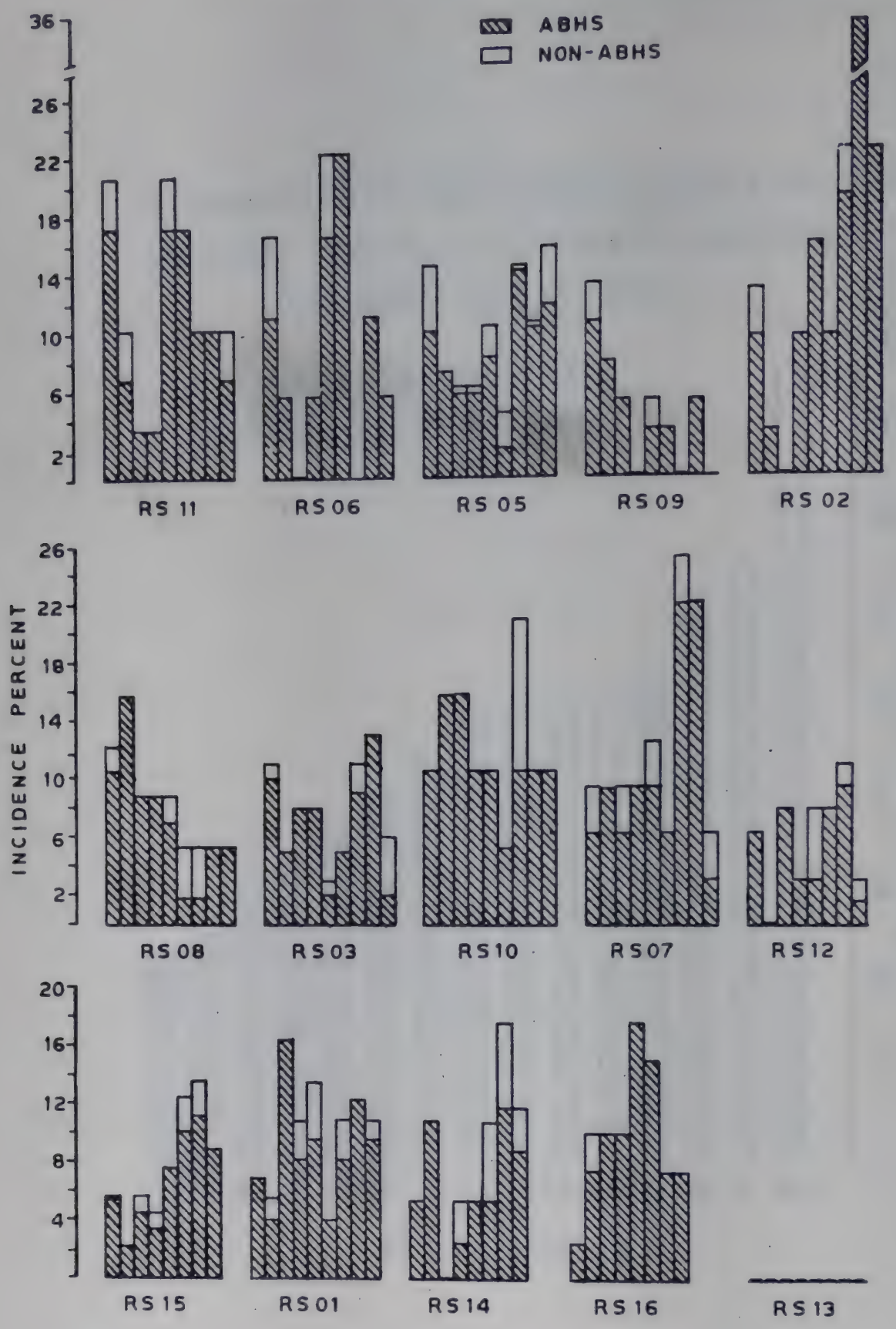
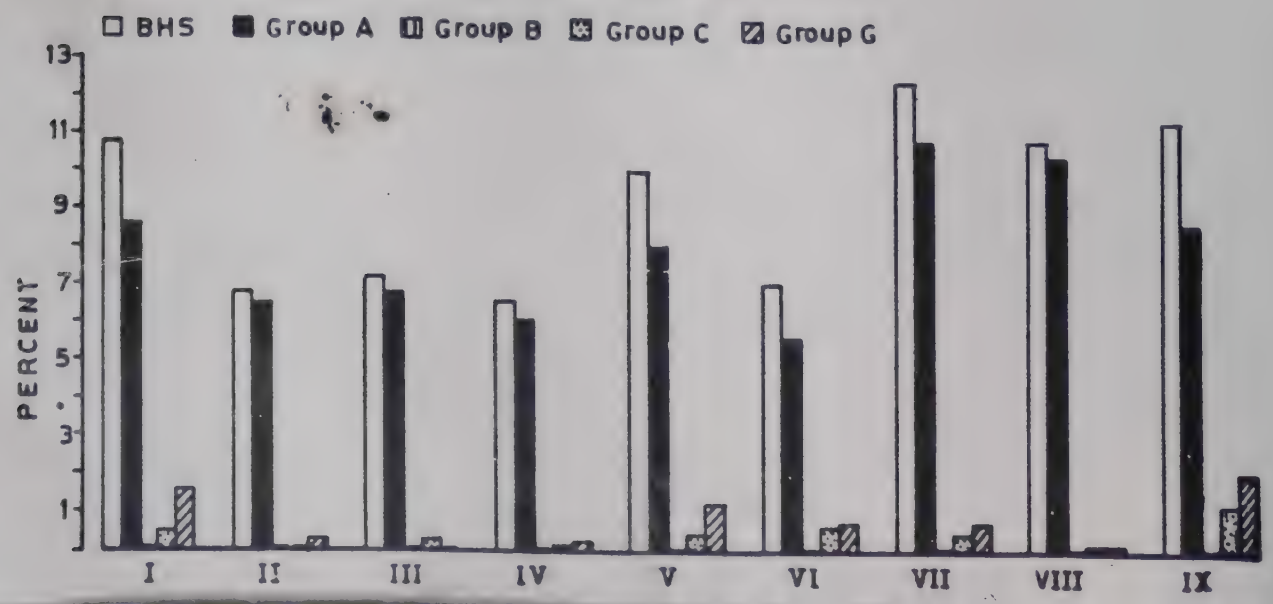



FIG 8.2

FIG 8.3

BHS AND THEIR ANTIGENIC GROUPS FROM PYODERMA LESIONS IN 923 CHILDREN OF RURAL SCHOOLS SEEN IN ALL 9 SURVEYS 1975 - 1978



(n=333)

* Significant Titre

ASO

1360
960
680
480
340 *
240
170
120
85
60

AH

≥2048
1024 *
512
256
128
64
32

ADNB

≥4800
320 *
2400
1600
1200
800 *
600
400
300
200
150
100
50

125

FIG 8.5

ASO, AH AND ADNB TITRES IN CLINICAL PYODERMA OF SCHOOL CHILDREN (n=353)

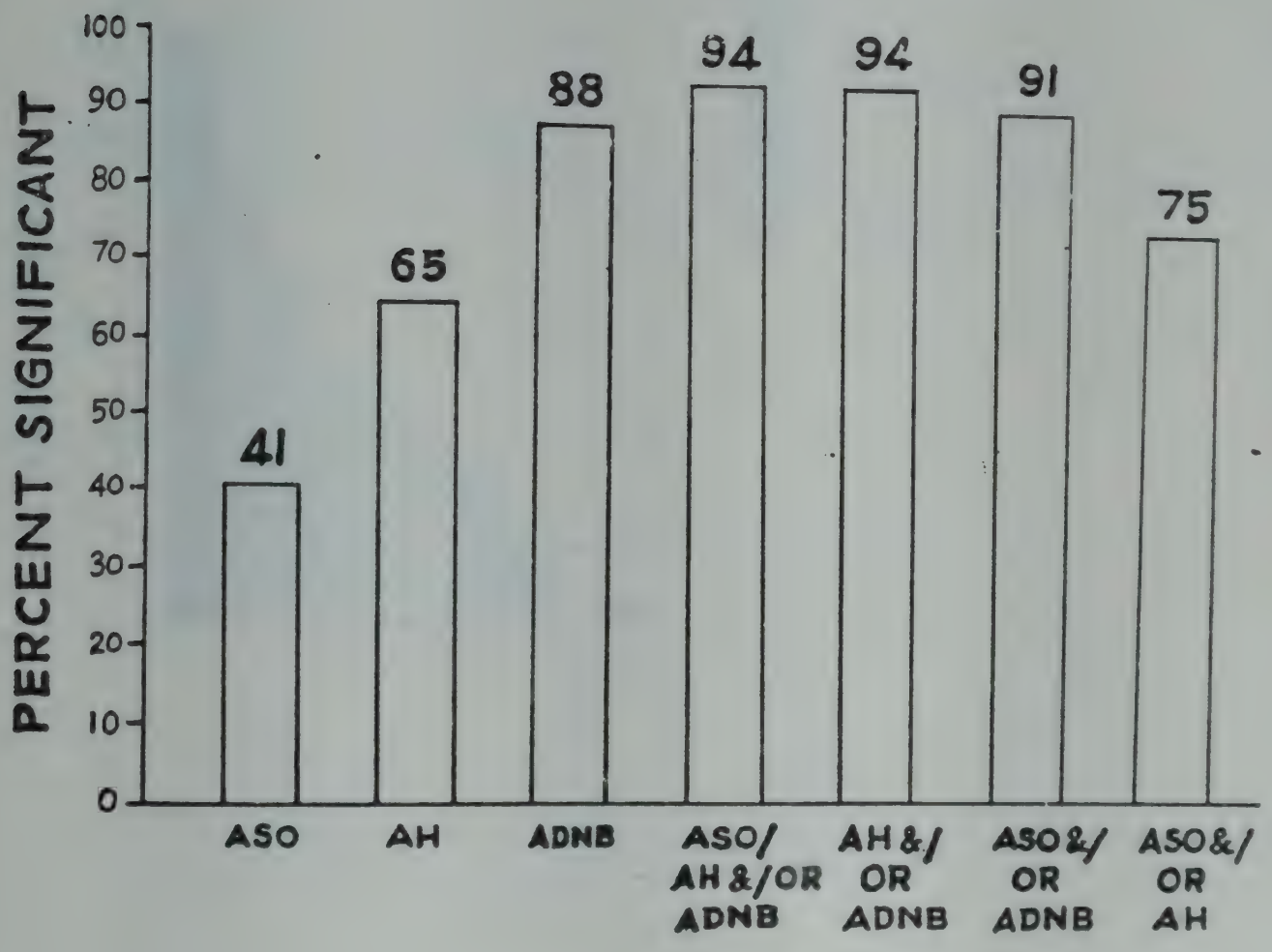
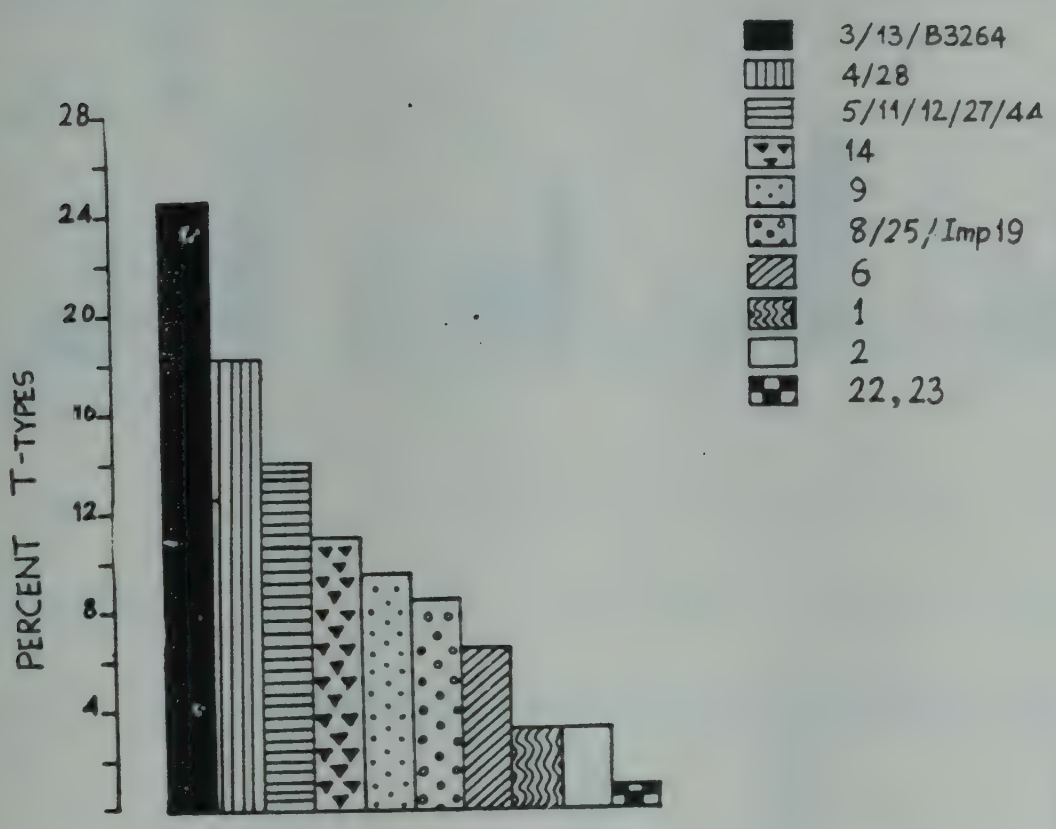
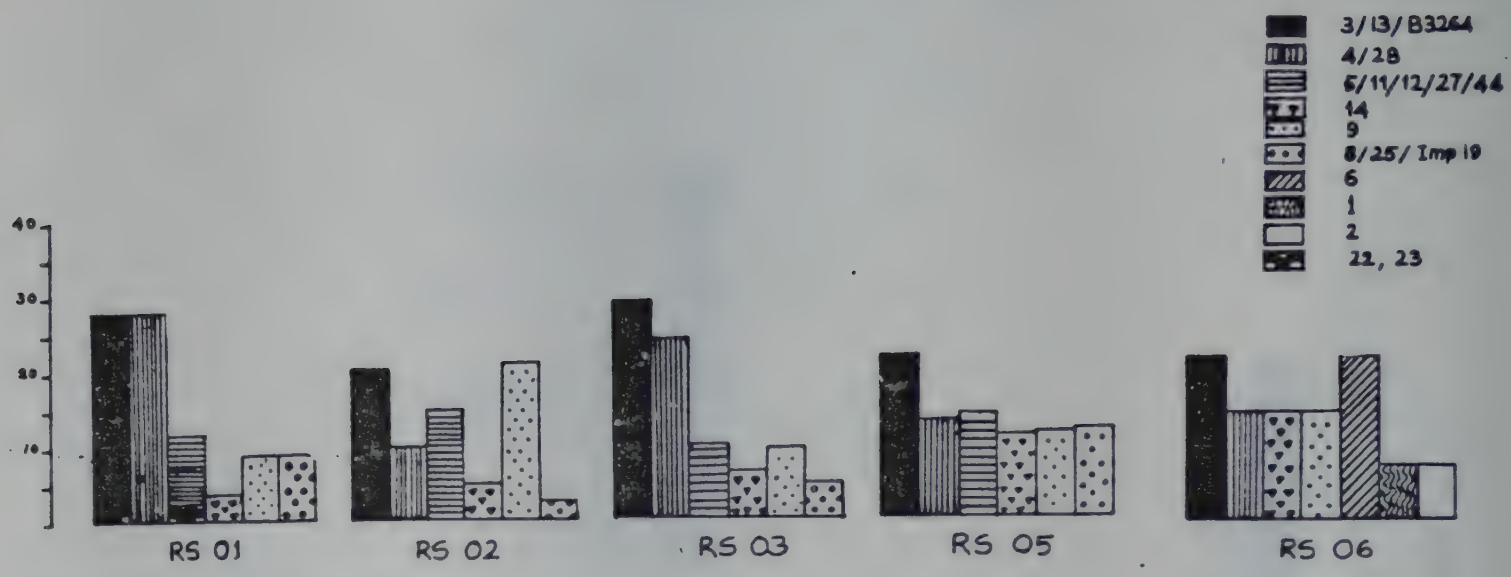


FIG 8.6

T-TYPES OF 632 ABHS STRAINS FROM SKIN LESIONS OF CHILDREN WITH PYODERMA SEEN IN 15 SCHOOLS.



# T-TYPES OF ABHS (632 STRAINS) FROM SKIN LESIONS OF CHILDREN WITH PYODERMA



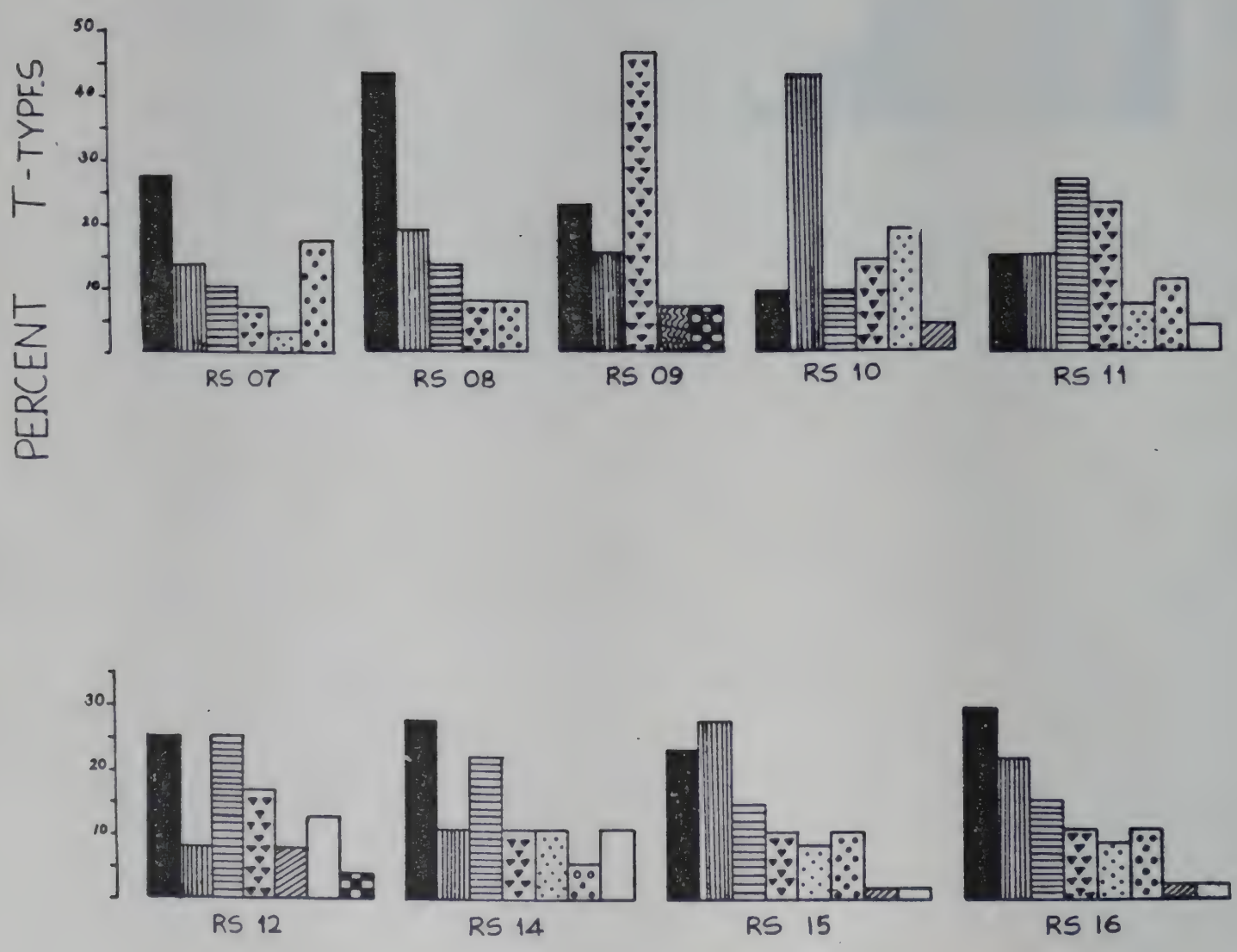
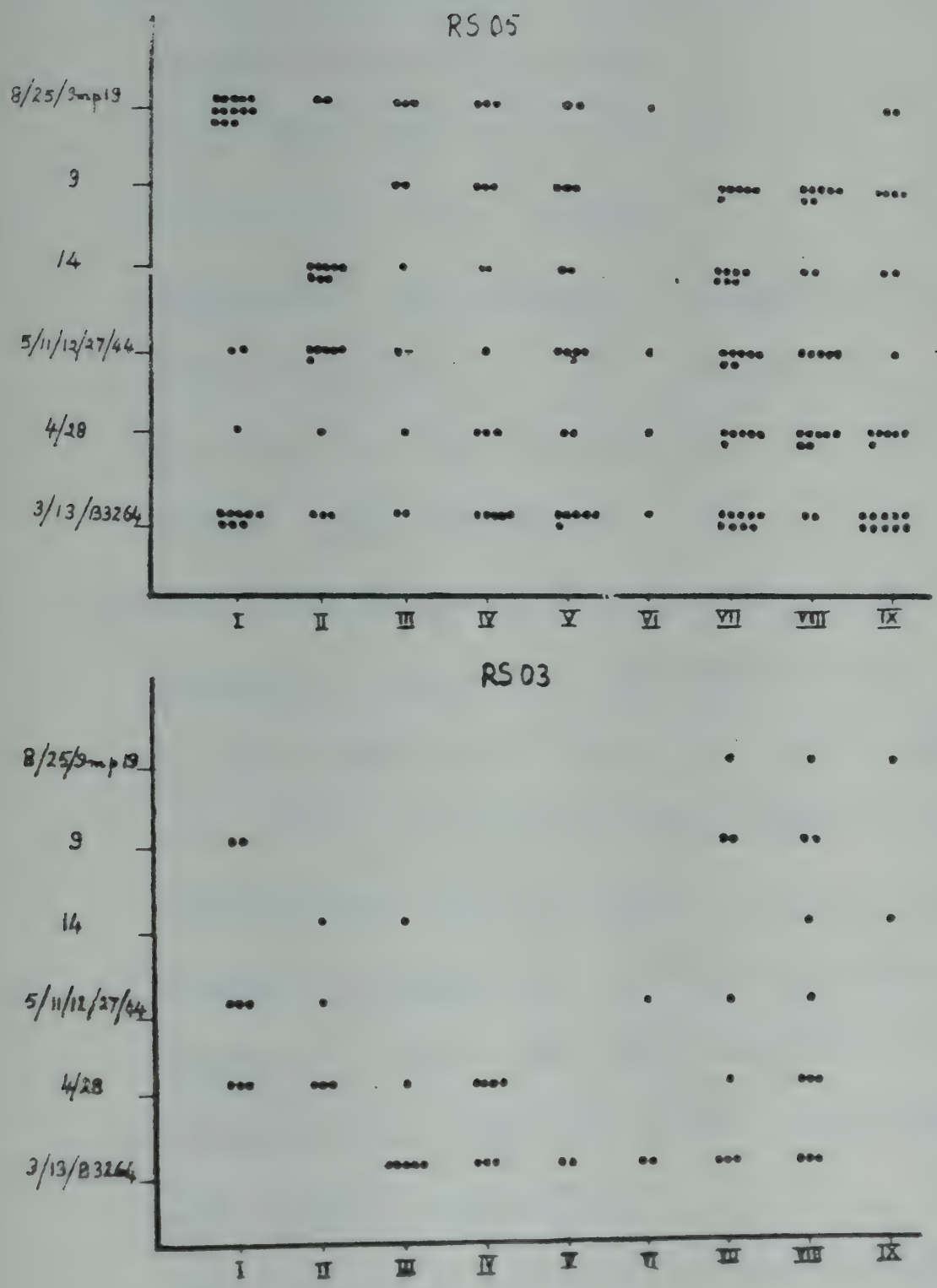




FIG 8·7

FIG 8.8



SURVEY-WISE DISTRIBUTION OF
T-TYPES OF ABHS
FROM PYODERMA LESIONS OF
CHILDREN OF TWO RURAL SCHOOLS.



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# CHAPTER 9

## STREPTOCOCCAL PYODERMA IN NON-SCHOOL GOING AND PRE-SCHOOL GOING CHILDREN

To determine the variation in prevalence and incidence of pyoderma, if any, in non-school going and pre-school children, a survey covering children from 0-15 years of age in the homes of all the villages was done. The numbers surveyed, village-wise, are given in Table 9.1. Their age-sex distribution is given in Table 9.2. A total of 1281 were pre-school children having almost equal sex distribution; 734 were non-school goers, 6-15 years of age.

Clinical streptoccal pharyngitis was seen in 9.6% but BHS isolation was done only in 1.0% and ABHS in 0.2% (Table 9.3), slightly lower than that seen in school goers (Refer Table 7.3). Streptococcal pyoderma was almost similar to that seen in school goers; clinical suspicion was found in 11.8%. BHS isolation in 8.1% and ABHS in 7.1% (Table 9.4 and Table 8.1).

Follow up studies were undertaken among pre-school goers in 4 villages in 1977. Streptococcal pharyngitis was least common (Table 9.5), whereas streptococcal pyoderma was similar (Table 9.6) to that seen in the school-goers (Refer Table 8.1) as well as in the non-school goers and pre-school goers screened among children seen at home (Table 9.3). However, the point prevalence (incidence ) of BHS and ABHS in pyoderma during the 2nd and 3rd survey tended to show a

decrease. This decrease in incidence was probably due to the impact of treatment, combined with lack of re-exposure, unlike the conditions found in the schools.

Among the T-types involved, type 3/13/B3264 was the most common. In pre-school goers, type 5/11/12/27/44 was the 2nd highest, whereas in non-school goers, type 8/25/Imp.19 was the 2nd most common and type 4/28 was the third most common in both groups( Table 9.7).

Thus the findings in non-school going and pre-school children were almost similar to those in the schools, except that streptococcal pharyngitis was very rare and was even less than that seen in school children; streptococcal pyoderma was as common in the non-school going and pre-school children as in the school-going children. However, the findings were different from those seen in school, in that treatment seemed to have some impact in the reduction of streptococcal pyoderma in the subsequent surveys. This reduction in the streptococcal pyoderma in the 2nd and 3rd survey must have been due to health care, lessening of injuries at home and lack of re-exposure to fresh cases, as opposed to the conditions found in the schools.

Scabies epidemic in V23 and its impact on streptococcal pyoderma in RS16

An epidemic of pyoderma following an epidemic of scabies occurred in V23 and its school RS16 in July 1977. Out of 71 subjects with clinical pyoderma examined from

the village, 41 (57.7%) had BHS. Of the BHS isolated, 92.7% were ABHS of which 31% belonged to T-type 3/13/B3264, 17.9% to T-type 8/25/Imp.19 and 15.4% to T-type 6. Higher nasal carriage of types 3/13/B3264 and 8/25/Imp.19 was found in these subjects as well.

The control of streptococcal pyoderma by prompt treatment of scabies with ointment, prompt prophylaxis with benzathine benzyl penicillin I.M., health education and hygiene appeared to be more effective in the homes than in the school-going population. No outbreak of post-streptococcal glomerulonephritis occured.

Table 9.1

AGE DISTRIBUTION OF NON-SCHOOL GOING CHILDREN SURVEYED AT HOME IN 13 VILLAGES

| VILLAGE CODE | AGE IN YEARS | | | |
|---|---|---|---|---|
| | 0-5 | 6-10 | 11-15 | 0-15 |
| V02 | 183 | 40 | 75 | 298 |
| V03 | 19 | 1 | 10 | 30 |
| V04 | 143 | 12 | 31 | 186 |
| V07 | 204 | 23 | 31 | 258 |
| V08 | 55 | 4 | 18 | 77 |
| V09 | 50 | 10 | 11 | 71 |
| V10 | 95 | 20 | 55 | 170 |
| V11 | 76 | 29 | 48 | 153 |
| V21 | 137 | 35 | 62 | 234 |
| V22 | 43 | 16 | 15 | 74 |
| V23 | 124 | 18 | 35 | 177 |
| V24 | 30 | 26 | 21 | 77 |
| V25 | 122 | 22 | 66 | 210 |
| TOTAL | 1281 | 256 | 478 | 2015 |

TABLE 9.2

AGE-SEX DISTRIBUTION OF NON-SCHOOL GOING CHILDREN SURVEYED AT HOME

| AGE IN YEARS | MALE | FEMALE | TOTAL | |
|---|---|---|---|---|
| | | | NO. | % |
| 0-5 | 663 | 618 | 1281 | 63.6 |
| 6-10 | 94 | 162 | 256 | 12.7 |
| 11-15 | 168 | 310 | 478 | 23.7 |
| TOTAL | 925 | 1090 | 2015 | 100.0 |

TABLE 9.3

PREVALENCE OF CLINICAL PHARYNGITIS, BHS AND ABHS RECOVERY FROM NON-SCHOOL GOING CHILDREN SEEN AT HOME

| VILLAGE CODE | NO. SEEN | CLINICAL CASE | | BHS | | ABHS | |
|---|---|---|---|---|---|---|---|
| | | NO. | % | NO. | % | NO. | % |
| V02 | 298 | 35 | 15.0 | 2 | 0.7 | 1 | 0.3 |
| V03 | 30 | - | - | - | - | - | - |
| V04 | 186 | 34 | 18.3 | 2 | 1.1 | - | - |
| V07 | 258 | 19 | 7.4 | 3 | 1.2 | - | - |
| V08 | 77 | 2 | 2.6 | - | - | - | - |
| V09 | 71 | 12 | 16.9 | 1 | 1.4 | 1 | 1.4 |
| V10 | 170 | 31 | 18.2 | 4 | 2.3 | 1 | 0.6 |
| V11 | 153 | 39 | 25.5 | 6 | 3.9 | 1 | 0.7 |
| V21 | 234 | 7 | 3.0 | 1 | 0.4 | - | - |
| V22 | 74 | 1 | 1.4 | - | - | - | - |
| V23 | 177 | 9 | 5.1 | - | - | - | - |
| V24 | 77 | - | - | - | - | - | - |
| V25 | 210 | 5 | 2.4 | 1 | 0.5 | 1 | 0.5 |
| TOTAL | 2015 | 194 | 9.6 | 20 | 1.0 | 5 | 0.2 |

TABLE 9.4

PREVALENCE OF CLINICAL PYODERMA, BHS AND ABHS RECOVERY FROM NON-SCHOOL GOING CHILDREN SEEN AT HOME

| VILLAGE CODE | NO. SEEN | CLINICAL CASES | | BHS | | ABHS | |
|---|---|---|---|---|---|---|---|
| | | NO. | % | NO. | % | NO. | % |
| V02 | 298 | 52 | 17.4 | 37 | 12.4 | 32 | 10.7 |
| V03 | 30 | 3 | 10.0 | 2 | 6.7 | 2 | 6.7 |
| V04 | 186 | 15 | 17.4 | 12 | 13.9 | 10 | 11.6 |
| V07 | 258 | 37 | 14.3 | 26 | 10.1 | 21 | 8.1 |
| V08 | 77 | 8 | 10.3 | 3 | 3.9 | 3 | 3.9 |
| V09 | 71 | 6 | 8.5 | 3 | 4.2 | 3 | 4.2 |
| V10 | 170 | 16 | 13.7 | 9 | 7.7 | 8 | 6.8 |
| V11 | 153 | 21 | 13.7 | 13 | 8.5 | 8 | 5.2 |
| V21 | 234 | 16 | 6.8 | 7 | 3.0 | 7 | 3.0 |
| V22 | 74 | 14 | 18.9 | 13 | 17.6 | 12 | 16.2 |
| V23 | 177 | 25 | 14.1 | 17 | 9.6 | 17 | 9.6 |
| V24 | 77 | 14 | 18.2 | 13 | 16.9 | 13 | 16.9 |
| V25 | 210 | 11 | 5.2 | 9 | 4.3 | 9 | 4.3 |
| TOTAL | 2015 | 238 | 11.8 | 164 | 8.1 | 145 | 7.2 |

TABLE 9.5

STREPTOCOCCAL PHARYNGITIS IN PRE-SCHOOL CHILDREN DURING 3 SURVEYS

| SURVEY | NO. SEEN | CLINICAL CASE | BHS % | ANTIGENIC GROUP A | B | C | G |
|---|---|---|---|---|---|---|---|
| I | 541 | 9 (1.7%) | – | – | – | – | – |
| II | 585 | 4 (0.7%) | – | – | – | – | – |
| III | 495 | 6 (1.2%) | 1 (0.2%) | – | 1 (0.2%) | – | – |

TABLE 9.6

STREPTOCOCCAL PYODERMA IN PRE-SCHOOL GOING CHILDREN IN 4 VILLAGES DURING 3 SURVEYS

| SURVEY | NO. SEEN | CLINICAL PYODERMA NO. | % | BHS % | ANTIGENIC GROUP PERCENT A | B | C | D |
|---|---|---|---|---|---|---|---|---|
| I | 541 | 79 | 14.6 | 8.1 | 7.4 | 0.2 | 0.2 | 0.4 |
| II | 585 | 66 | 11.3 | 6.3 | 4.8 | 0.3 | 0.5 | 0.7 |
| III | 495 | 56 | 11.3 | 5.1 | 2.6 | 0.2 | 1.4 | 0.8 |

TABLE 9.7

T-TYPES OF ISOLATES FROM NON-SCHOOL GOERS AND PRE-SCHOOL GOERS IN VILLAGE SURVEYS

| T-TYPES | NON-SCHOOL GOERS 0-15 YRS | | PRE-SCHOOL GOERS | |
|---|---|---|---|---|
| | NO. | % | NO. | % |
| 1 | 4 | 2.9 | - | - |
| 3/13/B3264 | 38 | 27.5 | 25 | 30.0 |
| 2 | 3 | 2.2 | 2 | 2.5 |
| 4/28 | 22 | 15.9 | 12 | 14.8 |
| 6 | 6 | 4.3 | 3 | 3.7 |
| 5/11/12/27/44 | 15 | 10.9 | 14 | 17.3 |
| 8/25/Imp.19 | 30 | 21.7 | 6 | 7.4 |
| 14 | 8 | 5.8 | 6 | 7.4 |
| 9 | 11 | 8.0 | 11 | 13.6 |
| 22,23 | 1 | 0.7 | 2 | 2.5 |
| TOTAL TYPABLE | 138 | 99.3 | 81 | 100.0 |
| UNTYPABLE | 1 | 0.7 | | |

# CHAPTER 10

# RHEUMATIC FEVER (RF) AND RHEUMATIC HEART DISEASE (RHD) IN RURAL SCHOOL CHILDREN IN KANIYAMBADI, SOUTH INDIA - PREVENTION & CONTROL, A SUCCESS STORY

## Introduction

The magnitude of the problem of rheumatic fever (RF) and rheumatic heart disease (RHD) ranges widely in different countries. RF and RHD have almost or completely disappeared in the affluent countries [1,2]. Even in the latter (where RF is thought to be controlled), pockets do still exist, with low socio economic standards, and consequent high incidence of RF and RHD. In the U.S.A., for example, RHD is reported to affect 100,000 children & 1.7 million adults, with over 13,000 deaths each year [3]. The W.H.O. has taken an active interest in coordinating activities during the past three decades, while, more concentrated activities have taken place in the developing countries, for about two decades.

It is now well known that RF and RHD are most prevalent in children, particularly in the 5 to 15 years of age, and are most prevalent in the developing countries. While the very old notion held that RF and RHD was nonexistent in India [4,5] overwhelming evidence of, not only its existence, but of its high prevalence & incidence have also been widely recognised [6-11].

Though RHD is one of the most readily preventable chronic diseases, there was a lack of dependable and

accurate data on the actual prevalence & incidence of the disease in India. In this large subcontinent of India, with its wide variation in climatic & socioeconomic conditions, widely varying disease patterns also exist. What is true of the North, may not hold good in South, East or West of India. Hence, for accurate data, well controlled studies have to be undertaken in representative areas.

It was, with this idea, that we undertook a pilot study on streptococcal infections and sequelae among rural children, particularly school children, from 1973 to 1975 with financial support from W.H.O. and later I.C.M.R. The high prevalence of RF and RHD among the above population brought home the need for a more detailed and controlled surveillance study among rural school children around Vellore. The study reported here, on surveillance & control of RF & RHD in a defined population of school children in Southern India was made possible with the Netherlands Government Grant for 4 years from 1975 to 1978. A detailed report is given below.

## Materials & Methods

### Area chosen & subjects

Details on the area chosen in the Kaniyambadi block (Fig.2.2.) about 20 kilometres south of the Christian Medical College and Hospital (CMCH), Vellore, the 13 villages included and its population, the 15 schools (13 primary or elementary and 2 secondary or high schools) are given in Chapter 2. The population data, the meteriological

data & details of registration are also outlined therein.

Survey & Registration of subjects

All details of the children seen at the school were entered in the Family card (Appendix I A) and in the Member card (Appendix I B). All children were given a general physical examination; swabs were taken with non-toxic cotton swabs from the throat, nostrils & skin and smeared on the filter paper (FP) kits and transported to the base laboratory[12]. With the beta-hemolytic streptococci (BHS) isolated from any site, conventional procedures were followed for grouping[13,14] & typing[13,15]. Multiple streptococcal antibody studies, namely, antistreptolysin O (ASO)[16], antihyaluronidase (AH)[17], and anti D-Nase B (ADNB)[18] were carried out as detailed in Chapter 6. Children suspected of having any cardiac lesion were further examined by the Chief cardiologist, Dr. George Cherian, and all relevant laboratory investigations were carried out at the base hospital. Children, suspected as RF or RHD on preliminary examination, were registered on the Rheumatic Fever Card (Appendix I E). Laboratory investigations such as erythrocyte sedimentation rate (ESR), total & differential leukocyte count, C-reactive protein test (C-RP), electrocardiogram and chest x-ray were also done. All school children were examined regularly, every 3-4 months, and new cases of streptococcal infections, and RF & RHD were investigated fully, as already detailed.

Rheumatic Fever Registry

School children who were 'definite' and confirmed as RF or RHD, were included in a rheumatic fever registry (Appendix I E). Those who were unconfirmed were kept as "suspects" and entered on a "Suspect" list according to the recommendations of the World Health Organization. They were examined every 3 to 6 months for a period of 12-18 months; when confirmed as RF and RHD, they were entered on the RF Registry. If not confirmed, they were still kept as suspects and followed up for confirmation or were dropped out of the study. Registered patients were put on regular secondary prophylaxis, and examined every 3-4 weeks. Swabs from the throat, nose & skin lesions were taken for culture at every visit and blood samples obtained whenever permitted by the patient.

Prophylaxis & Health Education

All children with bacteriologically and/or serologically proved streptococcal tonsillitis, tonsillopharyngitis, or pyoderma were given primary prophylaxis of one injection of benzathine benzylpenicillin (1,200,000 IU in children over 5 years of age and 600,000 IU in children below 5 years), after sensitivity testing with 0.1 ml of a 100 IU/ml dilution of penicillin.

Children with confirmed RF or RHD were given secondary prophylaxis with benzathine benzylpenicillin, consisting of 1,200,000 IU intramuscularly or 600,000 IU if below 5 years, every 4 weeks in the early part of the study (1975).

Prophylaxis was given every 3 weeks from 1976 onwards, when it was known that 3 weekly prophylaxis was more effective than the 4 weekly schedule.

Health education of pupils, parents, and teachers was organized before the start of data collection, and at regular intervals during the study. This included talks and group discussions, and involved the use of visual aids and presentations of the data obtained in different schools.

## Results

The 13 villages belonging to the Kaniyambadi block consisted of 2499 families with 12670 members (Chapter 2 Table 2.1). 42.1% of members were children below 15 years of age.

### Registry of Children

All children who attended the 15 schools at any time were included in the study. Details are given in Table 10.1. Though only 2577 were registered during the 1st survey, new entries & drop-outs at different times continued throughout the four-year study. Thus a total of 3890 children were examined at any time during the study period. The registration of children, school-wise, is given in Table 10.2.

### Prevalence of RF & RHD in School Children

Among all the children examined in 15 schools, 2 cases of RF & 17 cases of RHD, a total of 19, were detected.

Schoolwise data is given in Table 10.3. The prevalence of RF was 0.5/1000, of RHD 4.4/1000, giving a combined prevalence of 4.9/1000. The prevalence rates among boys and girls differed, with 5.7/1000 among 2283 boys and 3.7/1000 among 1607 girls.

Details on the 27 RF & RHD patients during follow up

As seen in Table 10.4, only one of the 5 who had URI (18.5%) on follow up, had BHS in the throat which belonged to group G; none was isolated from nose. Of the 9 who had pyoderma (33.3%), 2 had BHS in the throat; 1 was group A, type T9 and another GGS; of the 2 isolates from the nose, both were group A and were T9; of the 4 Isolates from the skin, 2 were T9, 1 was T14 and 1 was 8/25/Imp 19.

However, among 629 cultures done on repeat follow up, 49 BHS were isolated from the throat; 3 were ABHS (1 each of T1, T12 & TB3264), 10 GBS, 14 GCS & 22 GGS. Of the 629 nasal cultures done, 2 ABHS (T1 & T12) & 3 GCS were isolated. Two skin swabs from healed skin lesion at pyoderma sites yielded 2 ABHS, 1 T28 & 1 8/25/Imp 19.

Incidence of RF & RHD in School Children

Regular surveys were conducted in each school at an interval of 3-4 months, upto a total of 9 surveys and till the end of 1978. In 1976, 4 new cases of RHD were detected giving an incidence rate of 1.7/1000/year; in 1977 there were 3 new cases of RHD and 1 of RF giving an incidence of 1.6/1000/year. No new cases were detected in the fourth

year of surveillance from January to December 1978. Thus, in all, there were a total of 27 cases of RF & RHD among 3890 school children belonging to 15 rural schools in Kaniyambadi Panchayat.

Details including Bacteriology in the 8 with incidence of RF & RHD

As seen in Table 10.5 among the 8 new cases of RF (1) and RHD (7), 6 (1 RF & 5 RHD), occured within 4 months and 2 RHD within 4 to 8 months of previous survey. While 4 had given a preceeding history of URI, an equal number had pyoderma; 3 patients had a history of both URI & Pyoderma and 3 with no recognised preceeding URI or Pyoderma.

Out of 118 cultures done during follow up, 1 patient had T12 isolation from throat & nose, 2 had GBS, 7 GCS and 13 had GGS. Interestingly, 4 patients had repeated carriership of groups C & G, 2 to 4 times each, Group G alternating with C or with groups C or G all the four times.

Prevalence & Incidence of RF/RHD in Pre & Non-school goers

Among 585 pre-school going children upto 5 years of age surveyed at home, once in 1976 & thrice in 1977, no case of RF or RHD was detected. Out of the 734 children in the 6-15 year age-group who did not attend school, 3 were found to have RHD giving a prevalence of 4.1/1000 when surveyed at home.

Thus, a total of 30 cases were registered from school going & non-school going children in this surveilloance programme. Among these, 60% were in 5-10 year age-group and 40% in the 11-15 year age-group. The youngest child was 5 years & 4 months old. The male to female ratio was 2:1.

A preceeding history of tonsillopharyngitis was given by 27% of affected rheumatic children, pyoderma with no pharyngitis by 7% and preceeding RF by 30% of the children. A familial or genetic predisposition was found only in one family (3.3%); one patient's sibling had RHD earlier.

Details of presenting clinical manifestations are given in Table 10.6. Murmur was present in 90% while carditis was present in 86.7%; arthritis was less common (6.6%) than arthralgia (10%); Chorea developed in one female child, who was on 4 weekly prophylaxis a week, prior to the next injection. Erythema marginatum & subcutaneous nodules were noted for their absence.

Patients compliance to Prophylaxis

During the first 6 months of the study in 1975, only 2 of the 6 confirmed cases of RF & RHD were on regular prophylaxis. However, with health education of children and the families and making the teachers understand their part in helping the school children, as well as constant follow up & free treatment offered at the primary health centre, school or home, 26 of the 27 registered (96.3%) patients were on regular prophylaxis in 1976. By 1977, three had moved away; 22 of the remaining 23, were on

'regular' prophylaxis (91.6%) and 1 each, on 'casual' and 'irregular' treatment. Thus the commpliance to prophalaxis was very good in the second & third year of our surveillance study.

## DISCUSSION

It is now widely recognised that RHD is the commonest form of cardiovascular disease accounting for more than 1/3 of all cardiac diseases encountered in major hospitals in India [1,19,20]. Reliable statistics are difficult to obtain because of various constraints such as lack of facilities for registration of cases, for reporting of deaths, and non-availability of autopsy findings etc. What is found in India is, to a large extent, true of other developing countries as well [1,19].

The pilot study we undertook initially with W.H.O. and later with I.C.M.R. support, during 1973-75 was continued as a full fledged surveillance programme for another four years with the aid of Netherlands Government. This probably was the first pioneering long term surveillance of streptococcal Infection & Sequelae to be undertaken in any rural community in India.

In recent years, some data have accumulated from other studies in India, particularly from Delhi and the multicentric survey initiated by the Indian Council of Medical Research [21] in Delhi, Agra, Hyderabad, Bombay and Alleppey. Results have been varied widely, partly attributable to the differences in methods of investigations; in some areas without even any microbiological investigations. The prevalence of RF & RHD in the above study varied from 1.8/1000 in Bombay, and 2.5/1000 in Kerala, to a high 11/1000 in New Delhi.

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The study area we included has a dry, humid climate for most of the year, with a brief rainy season and a very short & mild winter. In general, about 96% of the pupils belonged to the low-income, though there was differences in standards in different schools. Living conditions were unhygienic, and there was overcrowding both at shcools and home. These epidemiological factors contributed to the high rates of streptococcal infections, particularly pyoderma in these children [22]; however pharyngitis incidence was much lower than that of North India (Chapter 7).

The prevalence of RF & RHD in rural south Indian children in this study have been established as 4.9/1000 among school children and among non-school going children as 4.1/1000. The findings in the school goers & non-school goers were not statistically different.

The prevalence rates established in our studies both in the school going & non-school going rural children are much lower than the prevalence reported from Delhi and other developing countries [1,20]. This probably is a reflection more of the climatic conditions, as the socio-economic conditions are similar. In North India, there is a clear cut prolonged and very cold winter when pharyngitis is more common and incidence higher. The true streptococcal pharyngitis in Vellore in different studies have varied from 4.9% to 8.9% [23] and was lowest among the rural school children as found in this study (Chapter 7 - Table 7.3 & 7.4). RF is caused after repeated streptococcal infection

of the throat and the lower prevalence of RF & RHD in this area is therefore understandable. It may be added here that so far no evidence has been obtained for the rheumatogenicity of streptococci of pyoderma though pyoderma is highly endemic in this population (Chapter 8 - Table 8.4 & 8.5).

The incidence of RF & RHD was slightly higher in the 6-10-year age group, while the youngest child with rheumatic heart disease was aged 5 years, 4 months. The higher incidence in boys and the clinical manifestations were in agreement with the findings of other workers [20,21].

Silent RHD occurred in the 7 new cases within a period of 3-8 months (Table 10.5). This was consistent with the well recognized juvenile mitral stenosis found in India and other tropical countries [10-13]. This could be a reflection of repeated subclinical pharyngitis, or a result of the more prevalent streptococcal pyoderma. At least 7% of patients in this study had given preceeding history of streptococcal pyoderma with no obvious pharyngitis. During surveillance, 33.3% of the 27 cases developed pyoderma and only 18.5% developed pharyngitis. However, subclinical pharyngitis cannot be ruled out. Moreover, the possible pathogenic or a triggering role of groups C and G streptococci may also be kept in mind, especially because of the high carrier rate of group G streptococci (GGS) (Chapter 6) and a possible role of GGS in pharyngitis (Chapter 7). However, there is no direct evidence at present for such a hypothesis. It is

also worth mentioning that at least in South India it has been repeatedly shown in different studies that group A streptococcal pharyngitis is of very low incidence (nil to 0.9%) whereas streptococcal (ABHS) pyoderma is noted for a much higher incidence (Chapter 8). The repeated incidence of pyoderma infection by ABHS despite prophylaxis, unlike that of pharyngitis, is also noteworthy (Table 10.5). A comparison of the elimination of ABHS & BHS from throat of pharyngitis patients by primary prophylaxis (Fig. 7.4 & Table 7.4) with the high rates of incidence of pyoderma cases despite primary prophylaxis throughout the study is very striking (Fig. 8.8).

In Table 10.3, it is seen that the prevalence rate varied from school to school and no cases occured in 4 schools. The schools with high prevalence of RF & RHD belonged to villages with members in the lower income group; many of them were Harijans with joint family systems and with 6 to 20 members, in a family of non-vegetarians with less hygienic habits. The schools in general were very crowded, with some classes held under trees or with children squatting on floors. In RS13, which had the minimum number of pupils, was a purely Muslim school with children from large families and unhygienic habits; there was one case of RHD from this 40 member school. The overall prevalence of RF/RHD in the 11 schools varied from 3.6 to 25% per 1000.

On the other hand, the 4 schools, RS07, RS10, RS15 & RS16 with no cases of RF or RHD were situated in villages

which were habited generally by families of higher income groups and who were strictly vegetarians, with cleaner habits. These schools were generally of a better standard, with benches for seating all children who were also cleaner than of other schools.

In India, where, more than 80% of the population resides in villages, it is only fitting that any health oriented and developmental programme should serve the rural population, if dramatic & well worth results are to be obtained. The impact, when felt in the rural community, will spread among nearby communities, by word of mouth. Changes will be adopted, if results are good and perceptable and particularly, if monetary outlay is less. This was our experience in the streptococcal surveillance that we carried out during 1975-1978.

It was seen that the compliance to treatment of pharyngitis & pyoderma aiming at primary prophylaxis of RF & PSGN also neared 90 to 100% from the 4th survey onwards (Table 7.7 & 8.12). The compliance to secondary prophylaxis of RF & RHD was also very good.

Primary prophy laxis in any country is ideal , so that RF can be prevented. But in our large country with about 800 million population, of whom 80% or more reside in the villages, it is not feasible to cover all with primary prophylaxis. Hence, it is fitting that W.H.O. & I.C.M.R. are stressing more on the prevention of recurrence of RF/RHD, with its high morbidity & mortality.

Primary prevention of RF is difficult, noneconomical and probably an unattainable goal in rural areas, with under-educated and low income group population. With poor communication facilities and understaffed health services, the problem is even greater. Case findings and surveillance in schools, particularly higher elementary schools, are probably more rewarding.

Teachers as well as health visitors or field workers, who are provided with high level of technical information could be made use of, in health education. Visual aids such as posters, flannel and demonstration cards and graphs with actual informations on the prevalence of streptococcal infection, RF or PSGN in the local school or neighbouring villages and schools could be fed to the population in the form of a story, as we did in our surveillance study. The importance of case findings, proper investigations and need for primary and secondary prophylaxis must be impressed on them. Education of the parents, teachers and pupils should be an important part of the control of RF in the community. In a developing country like India, there are many constraints in the control of rheumatic fever. Other competing priorities such as infectious diseases, malnutrition, scarcity of trained personnel, non-availability ot penicillin and lack of funds, are some of the major constraints in instituting regular prophylaxis. From our experience with this group of rheumatic fever and rheumatic heart disease patients, we feel that compliance at

long term follow up and prophylaxis will be possible only if the services are gratuitous. By offering free treatment and health education,we were able to achieve regular prophylaxis in 82.4% of registered patients. For the first time in India, we were able to prevent incidence of rheumatic fever in a defined population by continuous surveillance of streptococcal infections and primary prophylaxis in the 15 rural schools.

Thus our study on the prevention of RF was a success story. With the cooperation of clinicians, microbiologists and epidemiologists, and better health education of children, parents and teachers as well as by offering free treatment, it will be possible to lower the incidence, mortality and morbidity of RF and RHD in India, as it has been achieved in the affluent countries.

In the final analysis, this can best achieved by the cooperation of population at large and their change in attitude to health priorities which will bring about a perceptible change in the incidence of RF and RHD.

TABLE 10.1

DATA ON SCHOOL CHILDREN INCLUDED IN THE STUDY

| | NUMBER | PERCENT |
|---|---|---|
| SCHOOL SURVEYED | 15 | |
| ELEMENTARY SCHOOLS (4-15 YEARS) | 13 | |
| HIGH SCHOOLS (6-16 YEARS) | 2 | |
| CHILDREN SURVEYED AT ANY TIME | 3890 | |
| MALE | 2283 | 59 |
| FEMALE | 1607 | 41 |
| CHILDREN SEEN IN FIRST SURVEY | 2577 | |
| MALE | 1541 | 60 |
| FEMALE | 1036 | 40 |

TABLE 10.2

DATA ON CHILDREN SCHOOL-WISE

| SCHOOL CODE | NUMBER AT 1ST SURVEY | NUMBER SEEN AT ANY TIME |
|---|---|---|
| RS 01 | 154 | 248 |
| RS 02 | 121 | 181 |
| RS 03 (HIGH SCHOOL) | 415 | 564 |
| RS 05 (HIGH SCHOOL) | 606 | 889 |
| RS 06 | 86 | 166 |
| RS 07 | 117 | 199 |
| RS 08 | 135 | 215 |
| RS 09 | 100 | 158 |
| RS 10 | 83 | 119 |
| RS 11 | 114 | 189 |
| RS 12 | 179 | 244 |
| RS 13 | 20 | 40 |
| RS 14 | 125 | 195 |
| RS 15 | 226 | 363 |
| RS 16 | 96 | 120 |
| TOTAL | 2577 | 3890 |

TABLE 10.3

PREVALENCE OF RF & RHD IN SOUTH INDIAN SCHOOL CHILDREN

| SCHOOL CODE | NO. OF CHILDREN | RF | RHD | TOTAL | |
|---|---|---|---|---|---|
| | | | | NUMBER | PREVALENCE/ 1000 |
| RS 01 | 248 | 0 | 1 | 1 | 4.0 |
| RS 02 | 181 | 0 | 2 | 2 | 11.1 |
| RS 03 | 564 | 0 | 2 | 2 | 3.6 |
| RS 05 | 889 | 1 | 4 | 5 | 5.6 |
| RS 06 | 166 | 1 | 0 | 1 | 6.0 |
| RS 07 | 199 | 0 | 0 | 0 | 0 |
| RS 08 | 215 | 0 | 1 | 1 | 4.7 |
| RS 09 | 158 | 0 | 1 | 1 | 6.3 |
| RS 10 | 119 | 0 | 0 | 0 | 0 |
| RS 11 | 189 | 0 | 1 | 1 | 5.3 |
| RS 12 | 244 | 0 | 1 | 1 | 4.1 |
| RS 13 | 40 | 0 | 1 | 1 | 25.0 |
| RS 14 | 195 | 0 | 3 | 3 | 15.4 |
| RS 15 | 363 | 0 | 0 | 0 | 0 |
| RS 16 | 120 | 0 | 0 | 0 | 0 |
| TOTAL | 3890 | 2 | 17 | 19 | 4.9 |

TABLE 10.3

PREVALENCE OF RF & RHD IN SOUTH INDIAN SCHOOL CHILDREN

| SCHOOL CODE | NO. OF CHILDREN | RF | RHD | TOTAL | |
|---|---|---|---|---|---|
| | | | | NUMBER | PREVALENCE/ 1000 |
| RS 01 | 248 | 0 | 1 | 1 | 4.0 |
| RS 02 | 181 | 0 | 2 | 2 | 11.1 |
| RS 03 | 564 | 0 | 2 | 2 | 3.6 |
| RS 05 | 889 | 1 | 4 | 5 | 5.6 |
| RS 06 | 166 | 1 | 0 | 1 | 6.0 |
| RS 07 | 199 | 0 | 0 | 0 | 0 |
| RS 08 | 215 | 0 | 1 | 1 | 4.7 |
| RS 09 | 158 | 0 | 1 | 1 | 6.3 |
| RS 10 | 119 | 0 | 0 | 0 | 0 |
| RS 11 | 189 | 0 | 1 | 1 | 5.3 |
| RS 12 | 244 | 0 | 1 | 1 | 4.1 |
| RS 13 | 40 | 0 | 1 | 1 | 25.0 |
| RS 14 | 195 | 0 | 3 | 3 | 15.4 |
| RS 15 | 363 | 0 | 0 | 0 | 0 |
| RS 16 | 120 | 0 | 0 | 0 | 0 |
| TOTAL | 3890 | 2 | 17 | 19 | 4.9 |

TABLE 10.4

BACTERIOLOGY IN 27 PREVALENCE CASES OF RF, RHD ON REPEAT FOLLOW UP

| PRECEEDING HISTORY | NO. | CULTURE DONE | BHS ISOLATION | ANTIGENIC GROUPS | | | |
|---|---|---|---|---|---|---|---|
| | | | | A | B | C | G |
| U R I | 5 (18.5%) | | | | | | |
| THROAT | | 5 | 1 | – | – | – | 1 |
| NOSE | | 4 | 0 | – | – | – | – |
| PYODERMA | 9 (33.3%) | | | | | | |
| THROAT | | 9 | 2 | 1 (T9) | – | – | 1 |
| NOSE | | 9 | 2 | 2 (T9) | – | – | – |
| SKIN | | 9 | 4 | 4 (2 T9, 1 T 14, 1 8/25/Imp 19) | – | – | – |
| NORMAL | 629 | | | | | | |
| THROAT | | 629 | 49 (7.8%) | 3 (T1, T12, T B3264) | 10 | 14 | 22 |
| NOSE | | 629 | 5 | 2 (T1, T 12) | – | 3 | – |
| SKIN | | 2 | 2 | 2 (T 28, 8/25/Imp 19) | | | |

## TABLE 10.5

## DETAILS ON 8 NEW CASES OF RF, RHD DEVELOPED DURING SURVEILLANCE

| | NO. | DATA |
|---|---|---|
| **INTERVAL FOR OCCURRENCE:** | | |
| RF | 1 | < 4 months |
| RHD | 7 | 5= < 4 months |
| | | 2= 4 to 8 months |
| **PRECEEDING HISTORY:** | | |
| PRECEEDING U.R.I. & JOINT PAINS | 2 | ( 1 HAD PYODERMA WHEN SEEN EARLIER – SKIN HAD T 1 ) |
| U.R.I. & PYODERMMA | 2 | |
| PYODERMA | 1 | ( HAD T28 EARLIER FROM SKIN) |
| NIL RELEVANT | 3 | |

**BACTERIOLOGY:**

| SITE | NO. OF TIMES CULTURED | NUMBER POSITIVE | ANTIGENIC GROUPS ISOLATED | | | |
|---|---|---|---|---|---|---|
| | | | A | B | C | G |
| THROAT | 118 | 23 | 1 ** | 2 | 7 @ | 13 @ |
| SKIN | 104 | – | – | – | – | – |
| NOSE | 115 | 1 | 1 ** | – | – | – |

* 7/8 WERE ON 'REGULAR' PROPHYLAXIS

** SAME PATIENT WITH T 12 IN THROAT & NOSE

@ FOUR PATIENTS HAD REPEATED GROUP C &/OR GROUP G CARRIERSHIP, 4 TIMES EACH.

TABLE 10.6

CLINICAL MANIFESTATIONS IN 30 RHEUMATIC FEVER AND RHEUMATIC HEART DISEASE PATIENTS IN SOUTH INDIA

| MANIFESTATION | NUMBER | PERCENTAGE |
|---|---|---|
| MURMER | 27 | 90.0 |
| CARDITIS | 26 | 86.7 |
| MITRAL REGURGITATION (MR) | 20 | 66.7 |
| MITRAL STENOSIS (MS) | 6 | 20.0 |
| AS AND MR | 3 | 10.0 |
| AORTIC REGURGITATION (AR) | 3 | 10.0 |
| AR, MS, AND MR | 1 | 3.3 |
| FEVER | 5 | 16.6 |
| ARTHRALGIA | 3 | 10.0 |
| ARTHRITIS | 2 | 6.6 |
| CONGESTIVE CARDIAC FAILURE | 1 | 3.3 |
| CHOREA | 1 | 3.3 |
| ERYTHEMA MARGINATUM | 0 | 0 |
| SUBCUTANEOUS NODULE | 0 | 0 |

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# CHAPTER 11

## EPIDEMIOLOGY OF STREPTOCOCCAL INFECTIONS AND SEQUELAE IN RURAL SOUTH INDIAN SCHOOL CHILDREN

Streptococcal infection of the upper respiratory tract (Pharyngitis), and of the skin (Pyoderma) as well as their sequelae, rheumatic fever (RF), rheumatic heart disease (RHD) and Acute Glomerulonephritis or post-streptococcal glomerulonephritis (PSGN), are world-wide problems. Their prevalence and incidence, however, have varied widely in different countries and in different parts of the same country [1-5].

Of these, the most common and singularly important problem is pharyngitis as it is a disease common in all countries, with varying incidence & severity and because the dreaded sequelae, RF. & RHD as well as PSGN can follow repeated attacks of pharyngitis [6-12].

On the other hand, pyoderma is generally considered as of secondary importance as it occurs in certain geographical situations and is nonexistent in other places. The only sequel that can follow it, is PSGN and to date no proof of the occurrence of RF & RHD has been shown, to follow pyoderma. Consequently, streptococcal infection of the skin has attracted very little attention till recently. However, research carried out in different parts of the world during nearly 2 decades have given greater recognition for streptococcal infection of the skin [1,13].

What are the differences in epidemiology of the two commonest forms of streptococcal infections in man and the sequelae they produce? Wannamaker[1] has brought out, in a masterly review, the clinical, laboratory, and epidemiological differences in these two types of infections by streptococci. Just as the prevalence and incidence of these infections vary in different countries, their epidemiology also may be different in certain respects. There are a multitude of factors[12] concerned with the environment, the organism and the host which interplay with one another and produce the effect, manifesting in a particular disease.

Environmental factors

Details on the area chosen is given in Fig. 11.1. Population data of the 13 villages & their 15 schools and all relevant general informations are given in Chapter 2. The low socio-economic standards with poor hygienic conditions, over-crowding at school and home (Fig. 2.8, 2.9) were also very important factors.

The general facilities and overall low level of living conditions among village population including children of rural schools were below average. For example, general conditions which existed in two of the largest schools in our series are given in Table 11.1. On an average, about 40 to 50 children were made to sit on floors of class rooms or under trees as schools often did not have adequate facilities (Fig. 2.9, 2.10).

The houses and schools were thatched huts (Fig. 2.6 & 2.7) with mud floors and were ill-ventilated. Cattle, dogs and other domestic pets were housed in close proximity in many houses (Fig. 2.6). Most families had single room houses (Fig. 2.5), and had joint family system with 6 to 20 members living in one house. Over-crowding and overall poor hygienic conditions were, thus, by far, the most important in the epidemiology of these infections, in this community.

Climatic conditions / Seasonal variations

The meteriological data collected daily from October 1976 to end of 1978 is represented in Fig. 11.2. Like in various other reports [1,12], climate was a very important factor in this part of South India also, which had influenced, and probably accounted for, the rarity of bonafide streptococcal pharyngitis and the higher prevalence of streptococcal pyoderma. This area had, for the most part of the year, a hot climate, part of which is hot & dry, and part, hot & humid climate, with rainy season or light winter.

The daily temperature varied between 20 to 28 $^{o}$C in the winter or cold months (November to February). In summer, the temperature varied between 28 to 42 $^{o}$C or even higher at times; often it was very hot & dry and lasted from March to June.

In normal controls, the BHS & ABHS carrier rate was highest in the colder months and least in the rainy season, at all sites, i.e. throat, skin & nose (Chapter 5 - Fig. 5.1

and Table 5.2). The overall prevalence of streptococcal pharyngitis was very low in this community, inspite of good bacteriological techniques such as Filter Paper transportation, as well as direct inoculation in the field and regular follow up undertaken by us. However, there did emerge a seasonal correlation. On reanalysing the season-wise & school-wise variations, the pattern of pharyngitis in the first survey was as shown in Fig. 11.3. The prevalence rate of BHS & ABHS pharyngitis were high in the winter or cold months in 4 schools surveyed followed by the rainy and humid months in a few schools; however, in the very hot & dry summer months, BHS pharyngitis rate was very low or absent. There were no isolations of ABHS in the 5 schools surveyed in summer. Hence, the cold or winter & rainy months were found to favour pharyngitis in Vellore as is common in most temperate climates [1,15,16]. During repeat surveys, the streptococcal load had decreased much in the throat because of primary prophylaxis and so no results were available for same schools in different seasons.

Because of the more common dry weather, the roads are dusty & dirty which, along with, lack of water supply add on the prevailing unhygienic conditions. The average rainfall was about 431 mm; often it was unpredictable. In 1977 October onwards, there was unusual rainfall with cyclonic storms. The area also suffered from periods of drought & famine. In general, the hot & humid seasons found in the relatively colder (winter) months and unpredictable rainy

months seen in Kaniyambadi was conducive to the high prevalence of streptococcal skin infections (Fig. 11.4). In general, streptococcal pyoderma was highly prevalent through out the year. In the hot & dry summer season on the other hand, the BHS and particularly ABHS pyoderma were much lower than the other two seasons. However, a number of other factors also come in to play, in modulation of pyoderma in a tropical climate.

It is widely accepted that streptococcal infections are prevalent in endemic proportions in communities of low economic conditions [17]. In our study among rural south Indian school children, as many as 90-95% of the subjects belonged to low income group (< Rs. 500/- month per family). It is also interesting to note that (90-92%) of all streptococcal infections in this community occurred in such low income groups. In an early study, however, Koshi & Benjamin [12] had found no difference in the prevalence rates between rural and urban children studying in the same school who were bracketed in the same low-income groups.

The role of insects as mechanical vectors of streptococci has been emphasized [1]. In our study, we found that most of the houses were heavily infested with insects such as cockroaches, house flies, mosquitoes and other biting insects. Infestations with head lice were very common among the children & impetigenous lesions on scalp & behind the ears were comon sites. Presence of scabies in children has been shown to be a very important pre disposing

factor for pyoderma. A high incidence of pyoderma associated with scabies was observed in one school (RS16) belonging to V24 in June - July 1977. The habits of children, the boys especially, walking bare foot and in not covering their body especially during hot and humid conditions usually exposed them to insect bites and mechanical injuries.

The age-sex findings were similar to other studies. Pyoderma was higher in boys and in the 6-10 year age group of school goers.

A large majority of about 96% of the pupils were from low-income group, though in a few schools, children from slightly higher socio-economic strata were attending. The absence of any RF or RHD in 4 schools, particularly in RS15 with relatively large strength is noteworthy (Table 11.2). Here was a specific example of how increase in their economic standards can influence their educational & hygienic outlook, lessen overcrowding & much better environmental factors, brought down the prevalence of streptococcal pharyngitis as well as RF/RHD in these schools. However interplay of many other factors seemed to determine the high prevalence of pyoderma in these 4 schools. Similarly, joint family system, with many members in crowded rooms with close contacts, as well as, respiratory infections with their sequelae, was a natural outcome found in many other schools where, the prevalence of RF, RHD varied between 3.6 to 25%. If recalculation is done

on prevalence in those 11 schools among its members, it was found to 6.2/1000.

Factors governing the organism

Few organisms can match the group A streptococci (GAS) in the variety of cellular & extracellular products which might conceivably contribute to the virulence or communicability of the epidemic strain [12,18]. To date there is no direct evidence that groups other than GAS can produce RF, RHD or PSGN. However, groups C & G have been incriminated in pharyngitis, food born infections of the upper respiratory tract etc. [19-21]. Group G & C have been repeatedly shown to be involved in pyodermas of the skin at Vellore [22-24]. The role of group G and possibly of group C in producing any of the sequelae is yet to be convincingly proved.

M and/or T-typing helps in the delineation of types commonly involved in different infections & sequelae [25]. The types differed in different countries during epidemics and interepidemic periods. Over the years, we have improved our T-typing ability with highly potent sera and with improvements in the techniques. We have been able to successfully type 98 to 99.6% of all ABHS isolates by T-typing, over the period. M-typing could be done only with limited number of sera we produced. In the present study in rural school children, the T-types commonly encountered in pharyngitis belonged to 3/13/B3264, 4/28, 5/11/12/27/44 complex and T14. In an earlier study [14], T types commonly

involved in pharyngitis were T14, T11/12, 3/13/B 3264 and in pyoderma, were 11/12. 3/13/B 3264 and T14 etc. From pyoderma, the commonest T-types encountered were 3/13/B 3264, 4/28,5/11/12/27/44, & T14 in that order. The 5th commonest type belonged to T8/25/Imp 19 while T-type 12 was noted for its rarity.

The isolates from throat, preceeding RF or RHD were too few for making any valid conclusions (Table 10.4). However, interesting findings of T-type variation from survey to survey and from school to school as well as continuation of certain T-types in a few consecutive surveys were met with, in the Vellore study (Chapter 8 - Fig. 8.8).

It is now recognised that M5 has more rheumatogenic tendency[26]. Though many probable nephritogenic streptococci were isolated from cases of pyoderma and from pharyngitis patients, they were not followed up as PSGN was not included in this study.

Host factors

Regarding predisposition of the host, many factors which are common with environmental factors such as low socio-economic conditions & poor hygienic conditions have already been mentioned. The effect of poor nutrition among various members in a large joint family with low income is also understandable.

The role of carriers cannot be over emphasized. The carrier rates in throat, nose, & skin have varied in different studies including ours[11] (Table 5 and Fig. 5.1).

Next to the high carrier rate of the throat came that of the skin, where ABHS was far predominant. This showed a direct epidemiological role on the higher rate of pyoderma. The seasonal variation in the carrier rate was particularly noteworthy and the higher carrier rate in winter coincided with the higher rate of pharyngitis and even of pyoderma. However as pharyngitis was far less prevalent than expected and as primary prophylaxis almost completely eliminated, the recurrent throat infections in children, the surveillance became a success.

On the other hand, in streptococcal pyoderma, treatment did not positively influence the recurrence. The pyoderma incidence was high throughout the 4-year surveillance, but irregular from survey to survey & from school to school. However, the prophylaxis in pyoderma patients who might have had pharyngitis also prevented new occurrence of RF/RHD or PSGN in the duration of the surveillance.

The genetic predisposition of hosts may also be kept in mind[27]. The HLA typing system in recent years have thrown some light in this direction, though we have no experience with it. We had detected one child having a sibling with RHD giving a rate of 3.3% in this group studied.

Thus, in conclusion, not one, but a multitude of factors are involved in the natural pathogenesis of the streptococcal infections and their sequelae in any community. Delineation of these factors are necessary for the understanding and prevention of the post streptococcal sequelae.

TABLE 11.1

DATA ON GENERAL CONDITIONS OF TWO RURAL SCHOOLS

| DETAILS | RS03 | RS05 |
|---|---|---|
| NAME OF VILLAGE | PENNATHUR | EDAYANSATHU |
| NO. OF CHILDREN SEEN | 564 | 889 |
| AREA OF SCHOOL | 144,000 sq.ft. | 7000 sq.ft. |
| PLAYGROUND | YES | HARDLY ANY |
| POND | YES | YES |
| WELL WATER | YES | YES |
| MUD POT WATER | YES | YES |
| TAP WATER | NO | NO |
| LATRINE FACILITIES | NO | YES |
| INSECTS | 97% | 99% |
| CATTLE, FOWLS | YES | YES |

TABLE 11.2

DATA ON STREPTOCOCCAL DISEASES IN THE 4 SCHOOLS WITH COMPARATIVELY BETTER SOCIO-ECONOMIC STANDARDS

| SCHOOL CODE | MEMBERS | PYODERMA | | PHARYNGITIS | | RF & RHD |
|---|---|---|---|---|---|---|
| | | BHS % | ABHS % | BHS % | ABHS % | PREVALENCE/ 1000 |
| RS07 | 117 | 10.3 | 6.8 | 9.4 | 2.6 | 0 |
| RS10 | 83 | 10.8 | 10.8 | 14.5 | 4.8 | 0 |
| RS15 | 226 | 7.5 | 7.1 | 0.4 | - | 0 |
| RS16 | 96 | 8.3 | 8.3 | 2.1 | - | 0 |

• Rural Schools ( RS ) Surveyed

Scale 3.5 cms = 3.0 kms

VELLORE PANCHAYAT UNION

RS Ø5

RS 11

RS Ø9

RS Ø3

RS Ø1

RS Ø2

RS Ø6

RS Ø8

RS 10

RS Ø7

ARCOT TALUK

RS 12

RS 13

RS 14

RS 16

RS 15

IKKATTU PANCHAYAT UNION

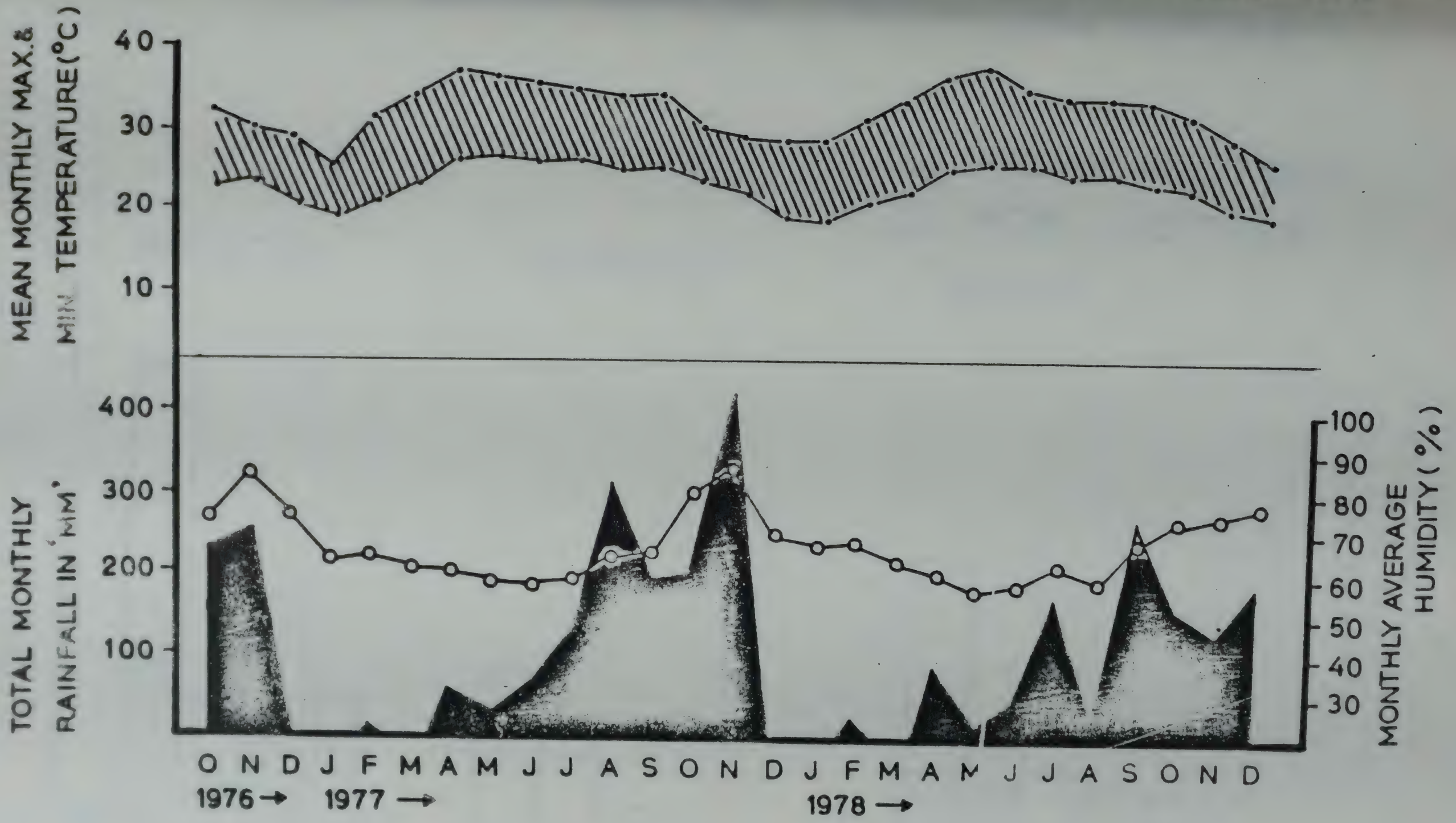
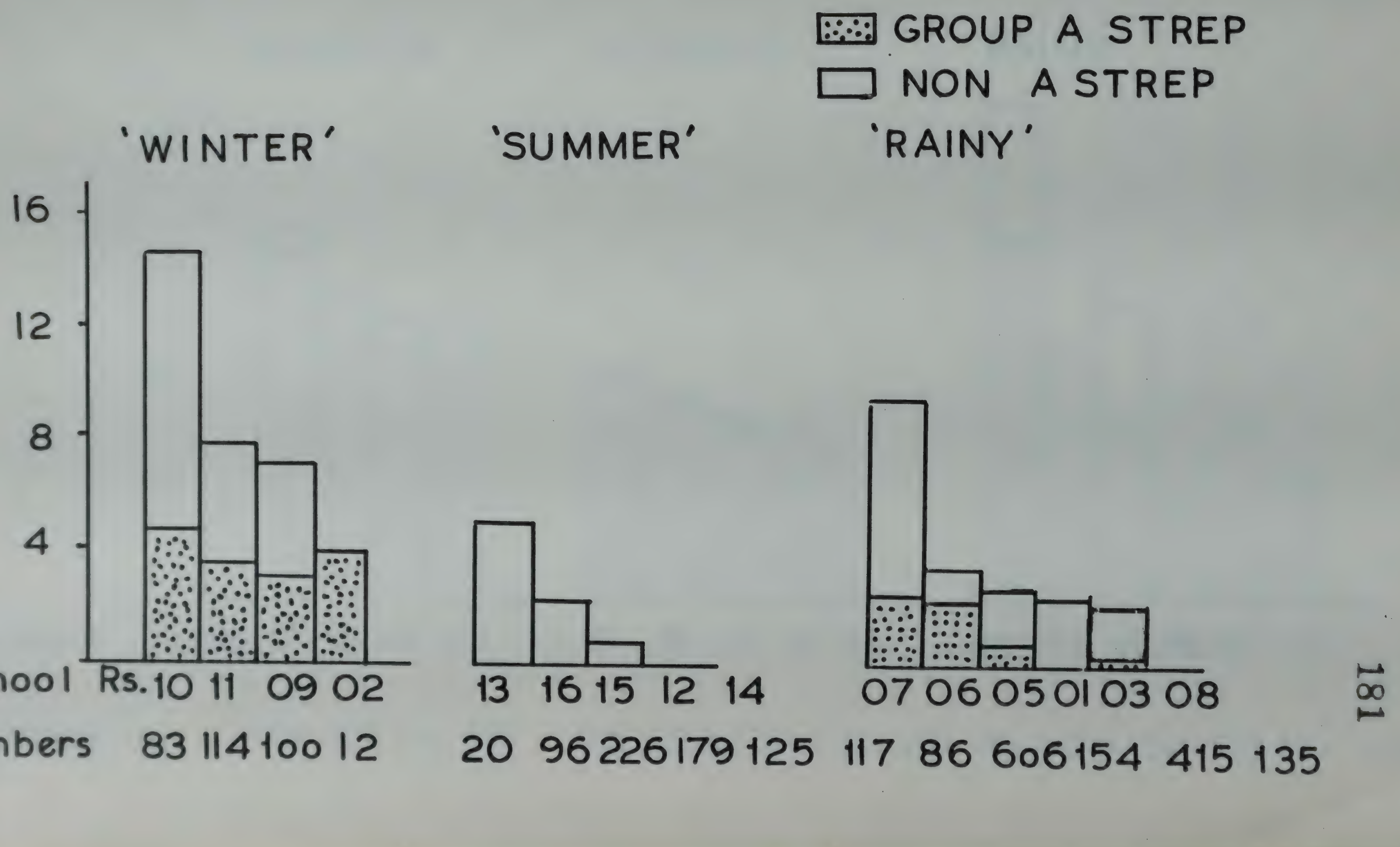

MEAN MONTHLY MAX.&
MIN. TEMPERATURE(°C)
40
30
20
10
TOTAL MONTHLY
RAINFALL IN 'MM'
400
300
200
100
100
90
80
70
60
50
40
30
MONTHLY AVERAGE
HUMIDITY(%)
O N D J F M A M J J A S O N D J F M A M J J A S O N D
1976→ 1977 → 1978 →

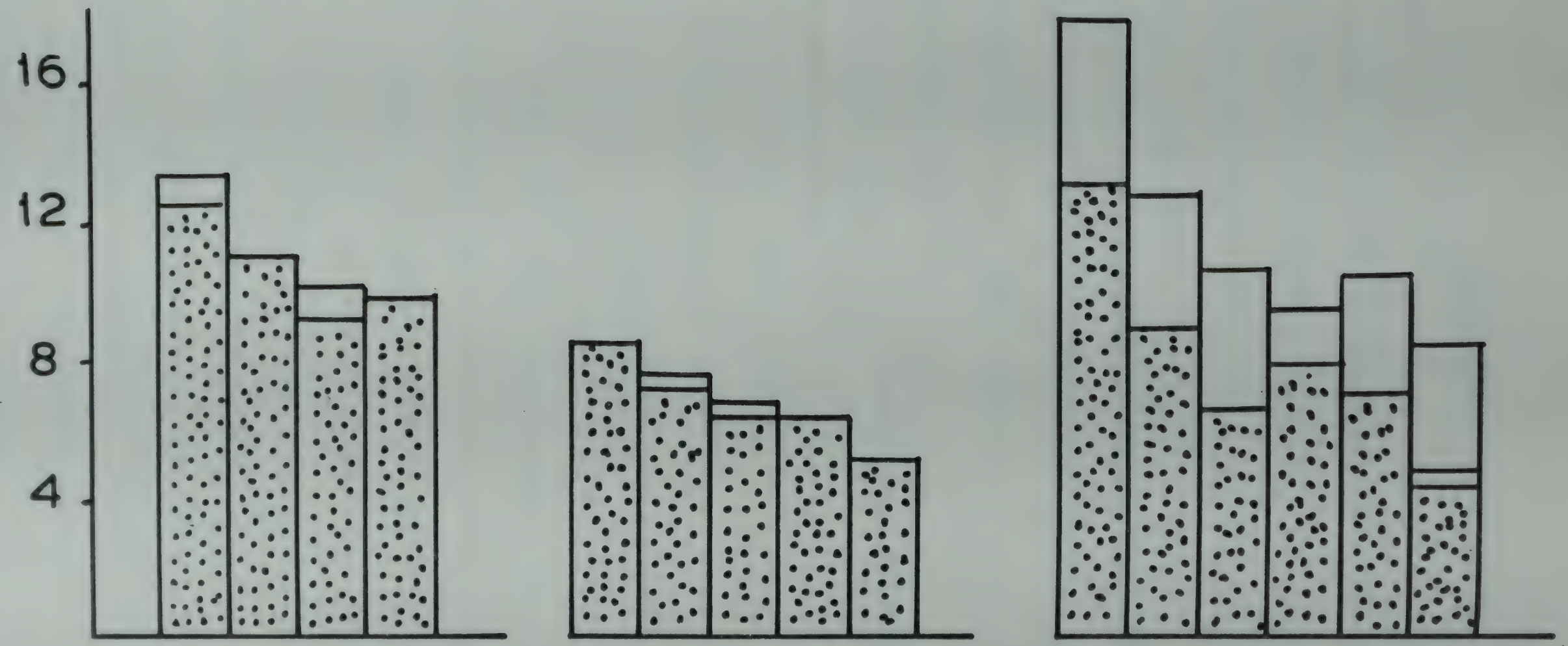
Fig II. 3. IN DIFFERENT SCHOOL _ PREVAENCE PERCENT



IN DIFFERENT SCHOOLS.

ABHS
NONA

'WINTER' 'SUMMER' 'RAINY'




| School | Rs | 11 | 10 | 09 | 02 | 16 | 15 | 12 | 14 | 13 | 06 | 05 | 01 | 08 | 07 | 03 | 182 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Members | 114 | 83 | 100 | 121 | 96 | 226 | 179 | 125 | 20 | 86 | 606 | 154 | 135 | 117 | 415 | | |

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# CHAPTER 12

# EPIDEMIOLOGY OF STREPTOCOCCAL PYODERMA IN A SOUTH INDIAN ORPHANAGE COMMUNITY

## Introduction

The prevalence of streptococcal pyoderma and its sequel, the post-streptococcal glomerulonephritis (PSGN) continues to be a problem of concern in the developing tropical countries and lesser developed parts of the affluent countries [1-5]. Several environmental factors such as climatic, hygienic and economic, are thought to interact with one another in the natural sequence of these conditions [5].

Reported literature on the real magnitude of the problem on pyoderma in India have been appearing in the literature [2-4,6]. The study reported here was undertaken to have a very thorough surveillance of a group of children in a small community with maximum cooperation. The bacteriological and serological aspects of pyoderma with very frequent follow up, among a group of orphanage children, 12 km from Vellore, South India is presented here.

## MATERIAL & METHODS

### Subjects and Method of Survey

Children residing at an orphanage under the care of Miss P. King, situated in Kasam, about 12 km north east of Vellore were surveyed every month from October 1976 to December 1978. All details of children with history of, or suspected of clinical pyoderma were [illegible]

Swabs for bacteriological studies were obtained from all children with clinical pyoderma. Contents from skin lesions, throat and nose from children with pyoderma were collected and transported to the laboratory by the filter paper method[7] and processed as previously reported[8a,8b]. Grouping of Beta hemolytic streptococci (BHS) and T-typing of group A beta hemolytic streptococci (GAS) were also carried out by standard procedures[10] using antisera prepared in this laboratory. Thirty strains of GAS, isolated from skin lesions, throat and nose of children with pyoderma, were sent to Dr. G. Colman, Public Health Laboratory Service, Colindale, London where M-typing was carried out.

Three antibody tests, namely antistreptolysin O (ASO), antihyaluronidase (AH) and antideoxyribonuclease B (ADNB) were carried out on sera collected from children with pyoderma. Due to non-cooperation from children, antibody studies had to be limited to certain surveys. All three tests were carried out by microtiter techniques[11-13] using reagents prepared in this laboratory and standardised against inter-national standards. AH testing was done with commercially available kits (Difco Laboratories, Detroit, Michigan, USA).

After confirming the streptococcal etiology, all children with pyoderma were given 600,000 units of benzathine benzyl penicillin (6 years of age) or 1,200,000 units ( >6 years of age).

Various weather data as reported from Vellore during the period of the study were collected from Meteriological Survey of India, Nungambakkam, Madras.

## RESULTS

Of eightynine children surveyed every month, 6 (6.8%) belonged to 3-5 year age group while majority (53.9%) belonged to 11-15 year age group. Male to female ratio was 1:0.7.

Among the 89 children seen for the first time during the study period, 9 (10.1%) had evidence of group ABHS pyoderma as defined by the isolation of ABHS from lesions (Table 12.1), giving a prevalence rate of 10.1%.

Month-wise analysis of clinical pyoderma, total BHS and GAS isolations are given in Fig. 1. The highest prevalence of pyoderma was observed in January 1978 followed by June 1977. Lowest prevalence was in August 1977 while there were no cases in August 1978.

During the study period, one or more episodes of ABHS pyoderma occurred in 64 children (Table 12.2); 36 children had only one episode while 28 had multiple episodes.

Age and sex-wise distribution of 120 episodes of pyoderma observed among 89 children showed that mean number of episodes was highest in males of 11-15 years of age group (Table 12.3).

Serological studies

Comparison of results of 3 antibody studies done on 83 sera from children with pyoderma showed that 48% had elevated ASO, 64% had elevated AH and 86% had elevated ADNB, titres. One or more antibodies were elevated in 95%.

Typing of GAS

T-typing: Of 133 strains of GAS isolated from skin lesions and throat and nose of children with pyoderma subjected to T-typing, all were typable. The typing patterns are given in Table 12.4. In the skin lesions, 3/13/B3264 were the most predominant (20.5%) followed by 4/28 and 5/11/12/27/44 (18.8%) each. T-9 followed next (17.1%) while 8/25/Imp 19 accounted for 10.3%. Other types were involved in 14.5%.

The seasonal distribution of various types is illustrated in Fig. 2. T-types, 3/13/B 3264 and 4/28 were present predominantly in 1st, 16th and 26th surveys with a complete absence for 4 to 5 months (12th to 16th surveys); however, these types along with 8/25/Imp 19 appeared during the latter part (16th to 19th and 22nd to 27th) of the survey.

T-9 was seen regularly from 7th to 11th surveys and then irregularly towards the latter half of the survey. T-14 was predominant in 9th and then again from 12th to 15th surveys. Type 5/11/12/27/44, especially T-12 appeared first during 13th survey and persisted together with T-11 till 24th survey. Other types were infrequently seen.

M-typing: Results of M typing done on 30 selected strains of GAS from skin lesions of children with pyoderma are given in Table 12.5. Of these, 16 (53.3%) could be M-typed. Seven recognised M-types & 2 provisional types were identified.

Epidemiological Factors

The incidence of pyoderma observed in the orphanage in relation to temperature, rain fall and humidity is compared in Fig. 3. The changes in incidence of pyoderma during different months showed corresponding changes in temperatures and/or humidity. In June 1977 and March & April 1978, the higher incidence could be related to increase in temperatures. The peaks of incidence in September and October 1977 as well as those of September and December 1978 was related to high humidity. However, the high incidence of pyoderma observed in January 1978 could not be related to any of the above three climatic factors.

Penicillin prophylaxis

Of 120 episodes of pyoderma observed during the study period, penicillin prophylaxis could be given in 117 cases (97.5%). Results of the effect of prophylaxis on the reacquisition of GAS on skin in 57 episodes of pyoderma are given in Table 12.6. It is seen that 4 of the 57 children (7.01%) who were followed for 4 weeks, had acquired GAS subsequently on skin during this period. Two of them were of the same type as those isolated from the original skin lesions and 2 were different.

## DISCUSSION

Available data on the morbidity of pyoderma in India, have indicated a high rate of prevalence [3-6], however all information have been based on hospital studies. Thus, the present study was undertaken to investigate, the bacteriology and serology of streptococcal pyoderma in a semiclosed community of children and to delineate the various epidemiological factors involved in its pathogenesis.

Reported literature from other parts of the world have shown prevalence rates of pyoderma varying from 10% to 87% depending on different methods of comparisons [15-17]. Due to our inability to screen children at more regular intervals as well as their frequent drop outs, the prevalence rate was calculated, out of the total children seen at the first visit. This was 10.1%.

GAS pyoderma is known to have a predilection for children of younger age group [16,15]. Bassett [18] observed a decline in pyoderma with age. The number of children below 5 years in the present study was too small to make any such comparison. However, a comparison of the other two groups, namely 5-10 years and 11-15 years showed a higher prevalence among the latter, especially so, in males.

Among GAS isolated from skin in the present study, the most predominant T-type was 3/13/B 3264, followed by 4/28, 5/11/12/27/44, 9 and 8/25/Imp 19, in that order (Table 12.4). Interestingly, 8/25/Imp 19, one of the commonest T-

types reported in most studies, was less commonly encountered in our study. In general, the T-patterns encountered continue to be the same as reported earlier from Vellore [19] and elsewhere [4,14-16] pointing to the similar epidemiologic patterns of pyoderma in different regions of the country irrespective of variations in climatic conditions and population studied.

The seasonal variations in the T-type distribution among this population was striking (Fig. 2). Such a distribution shows a seasonal distribution of a variety of corresponding M-types in the community. The results of preliminary M-typing (Courtesy: Dr. Col man, Colindale, London) done during this study confirm this observation (Table 12.5). It is interesting that despite the small number of strains typed, the percentage of typability was high. Furthermore, two were provisional types awaiting a number, designation and international ratification (Dr. G. Col man - Personal Communication). It is possible that some, as yet unknown types may also exist in this population.

The present study once again confirmed the relatively weak immune response to SLO in pyoderma [5]. Results of AH responses in pyoderma showed its usefulness as an adjunct in the serodiagnosis, as shown earlier [20]. Due to its better sensitivity and a higher magnitude of immune response, ADNB is the best serological test for confirming a diagnosis of GAS pyoderma [20]. The present study showed the superiority of

ADNB in the serodiagnosis of streptococcal pyoderma in the tropics.

A variety of factors are known to interact to favour the development of GAS pyoderma[5]. Regular monthly surveys conducted in the current study revealed the importance of a hot and/or humid tropical climate in the endemicity of pyoderma in this population. Various other factors related to pyoderma were strikingly similar to that discussed in Chapters 8 & 11. Despite a better standard of living conditions in the orphanage, the total prevalence of pyoderma remained high among the children. Carriage of BHS by the caretakers of the cottages was ruled out at each survey. Many of the children in the orphanage were attending different schools, some as far away as 12 kms. This could mean that they were exposed to different strains of GAS from other students at different times, making the disease endemic among these children.

Thus not one, but a variety of factors discussed above, were always prevalent in this population. As seen elsewhere in this report, better hygienic and general living conditions will go hand in hand with effective health education, in the prevention of this malady.

Several investigators have demonstrated the efficacy of penicillin G in the effective healing of skin lesions[25]. However, its effect on the reacquisition of GAS has been questionable. Penicillin prophylaxis and its impact on 57 children followed up every week for 4 weeks showed that

7.01% had acquired GAS on the skin within a 0-4 week period. (Table 12.6). Ferrieri et al[21] have shown that 18% of 78 children examined had new lesions within 5 weeks of receiving Bicillin. The 4 weekly follow-up in the present study showed that none of 57 children with pyoderma, who had been given penicillin prophylaxis, had repeated episodes within this period. Thus, benzathine penicillin was shown to give a protection for a period of atleast 4 weeks.

One important observation on the impact of prophylaxis was the absence of any new cases of PSGN or RF/RHD during the survey period of 27 months. A high percentage (99.7%) of prophylactic coverage could be the main reason for the absence of PSGN or RF/RHD during this period. This confirms that continued surveillance and prophylaxis of GAS pyoderma is contributory to the prevention of PSGN and RF/RHD in a community.

TABLE 12.1

PREVALENCE OF STREPTOCOCCAL PYODERMA

AMONG ORPHANAGE CHILDREN

| CATEGORY | NUMBER | PERCENT |
|---|---|---|
| TOTAL NO. OF CHILDREN SEEN: | 89 | |
| MALES .. | 51 | 57.3 |
| FEMALES .. | 38 | 42.7 |
| NO. OF CLINICAL PYODERMA | 11 | 12.4 |
| NO. OF ABHS PYODERMA | 9 | 10.1 |
| PREVALENCE RATE | 9 | 10.1 |

TABLE 12.2

EPISODES OF STREPTOCOCCAL PYODERMA AMONG

ORPHANAGE CHILDREN

| NUMBER OF EPISODES | AFFECTED CHILDREN | | TOTAL EPISODES |
|---|---|---|---|
| | NUMBER | PERCENTAGE | |
| 1 | 36 | 40.4 | 36 |
| 2 | 14 | 15.7 | 28 |
| 3 | 6 | 6.7 | 18 |
| 4 | 4 | 4.5 | 16 |
| 5 | 2 | 2.3 | 10 |
| 6 | 2 | 2.3 | 12 |
| TOTAL INFECTED | 64 | 71.9 | 120 |

TABLE 12.3

AGE-SEX DISTRIBUTION OF STREPTOCOCCAL PYODERMA AMONG ORPHANAGE CHILDREN

| CATEGORY | AGE IN YEARS | | | | | | TOTAL |
|---|---|---|---|---|---|---|---|
| | 4 - 5 | | 6 - 10 | | 11 - 15 | | |
| | M | F | M | F | M | F | |
| NO. OF CHILDREN SEEN | 3 | 3 | 21 | 14 | 27 | 21 | 89 |
| NO. OF ABHS | 1 | 4 | 32 | 19 | 50 | 14 | 120 |
| MEAN NO. OF EPISODES | 0.3 | 1.3 | 1.5 | 1.4 | 1.8 | 0.7 | 1.3 |

TABLE 12.4

T-TYPING PATTERNS OF ABHS FROM SKIN LESIONS, THROAT AND NOSE OF CHILDREN WITH PYODERMA

| T-TYPES | SKIN | | THROAT | | NOSE | |
|---|---|---|---|---|---|---|
| | NO. | % | NO. | % | NO. | % |
| 3/13/B3264 | 24 | 20.5 | 1 | 11.1 | - | - |
| 4/28 | 22 | 18.8 | 3 | 33.3 | 3 | 42.8 |
| 5/11/12/27/44 | 22 | 18.8 | 2 | 22.2 | - | - |
| 9 | 20 | 17.1 | 1 | 11.1 | 1 | 14.3 |
| 8/25/Imp 19 | 12 | 10.3 | 2 | 22.2 | 1 | 14.3 |
| 14 | 11 | 9.4 | - | - | 1 | 14.3 |
| 1, 2, 22, 23 | 6 | 5.1 | - | - | 1 | 14.3 |
| TOTAL | 117 | | 9 | | 7 | |

TABLE 12.5

CORRELATION OF T & M-TYPES OF 16 STRAINS ISOLATED FROM SKIN LESIONS OF CHILDREN WITH PYODERMA

| T-TYPE | NUMBER | M-TYPES * |
|---|---|---|
| 4 | 1 | 22 ** |
| 4 | 1 | 58 |
| 9 | 2 | 25 |
| 8/25/Imp 19 | 2 | 65 |
| 3 | 3 | TRIN NO. 2631 |
| 3/13/B3264 | 2 | 74 |
| 3/13/B3264 | 1 | 52 |
| 28 | 1 | 30 |
| 14 | 1 | 39 |
| 11 | 2 | TRIN POTTER C |

* TYPING DONE BY DR. G. COLMAN, PUBLIC HEALTH LABORATORY SERVICE, COLINDALE, LONDON, U.K.

** TYPING REACTION POSITIVE BY INHIBITION ALONE

TABLE 12.6

PROPHYLAXIS AND ITS IMPACT ON NEW ACQUISITION OF T-TYPES ON SKIN

| PERIOD OF SURVEY (1978) | STREPT. PYODERMA | INJECT @ GIVEN | T-TYPE +VE | |
|---|---|---|---|---|
| | | | SAME | DIFF. |
| JANUARY | 11 | 11 | – | – |
| FEBRUARY | 4 | 4 | – | – |
| MARCH | 6 | 6 | – | – |
| APRIL | 8 | 8 | – | – |
| MAY | 1 | 1 | – | – |
| JUNE | 5 | 5 | – | – |
| JULY | 7 | 7 | 2 | 1 * |
| AUGUST | – | – | – | – |
| SEPTEMBER | 4 | 4 | – | – |
| OCTOBER | 3 | 3 | – | – |
| NOVEMBER | 2 | 2 | – | 1 |
| DECEMBER | 6 | 6 | – | – |

@ BENZYL BENZATHINE PENICILLIN, 0.6 & 1.2 MILLION UNITS (IM) RESPECTIVELY TO PATIENTS AGED < 6 YEARS AND > 6 YEARS.

* ALSO HAD GROUP G DURING THE FIRST WEEKLY FOLLOW-UP

Fig. 1 MONTH-WISE DISTRIBUTION OF CLINICAL, BHS AND GROUP ABHS PYODERMA AMONG ORPHANAGE CHILDREN

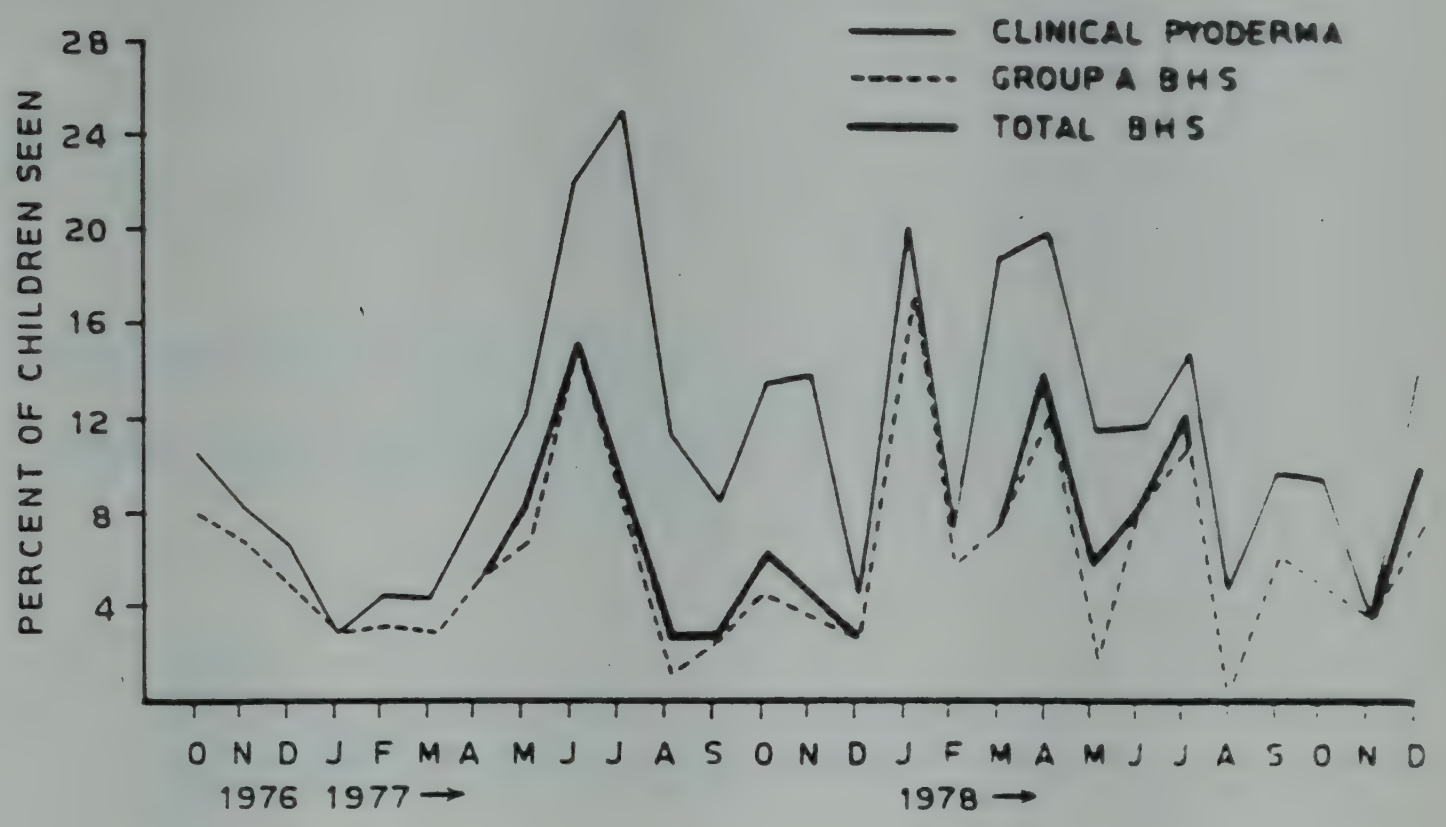



Fig. 2 T-TYPE DISTRIBUTION OF GROUP ABHS FROM SKIN LESIONS OF ORPHANAGE CHILDREN

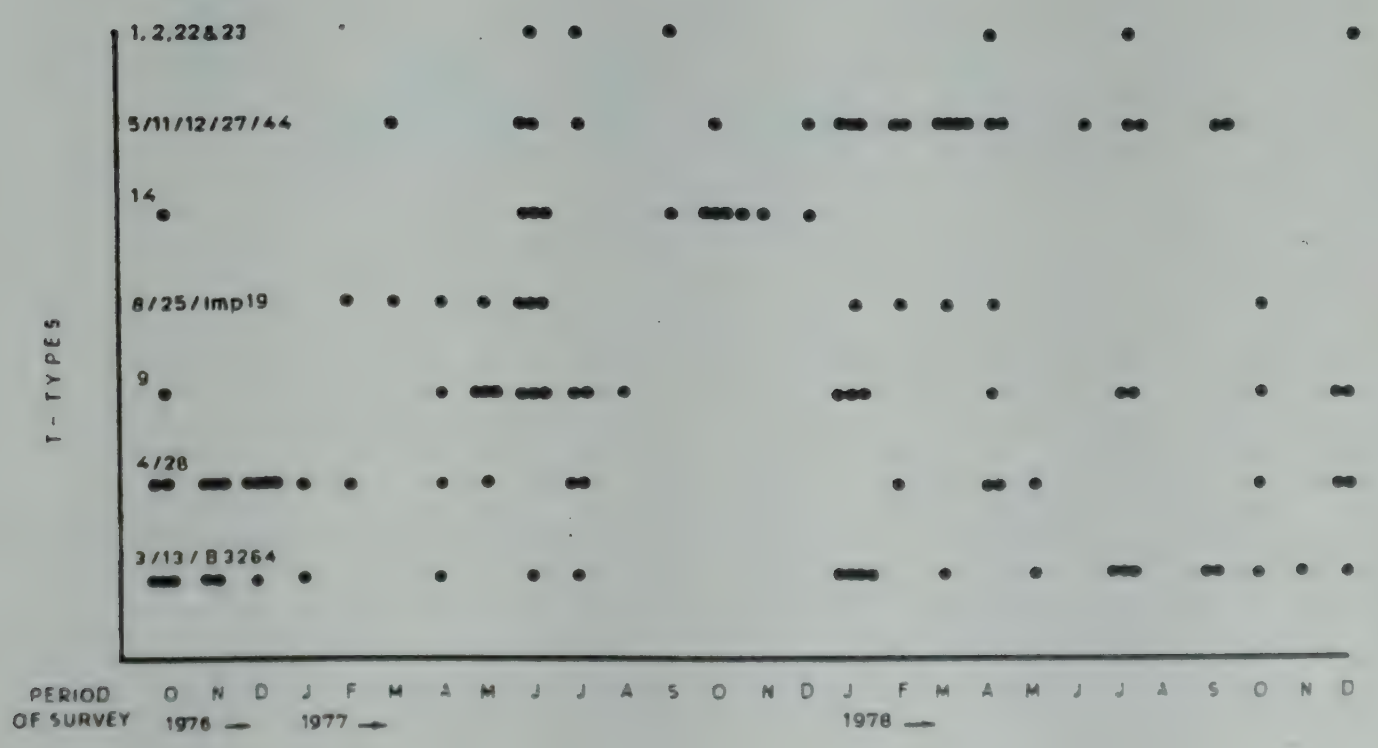
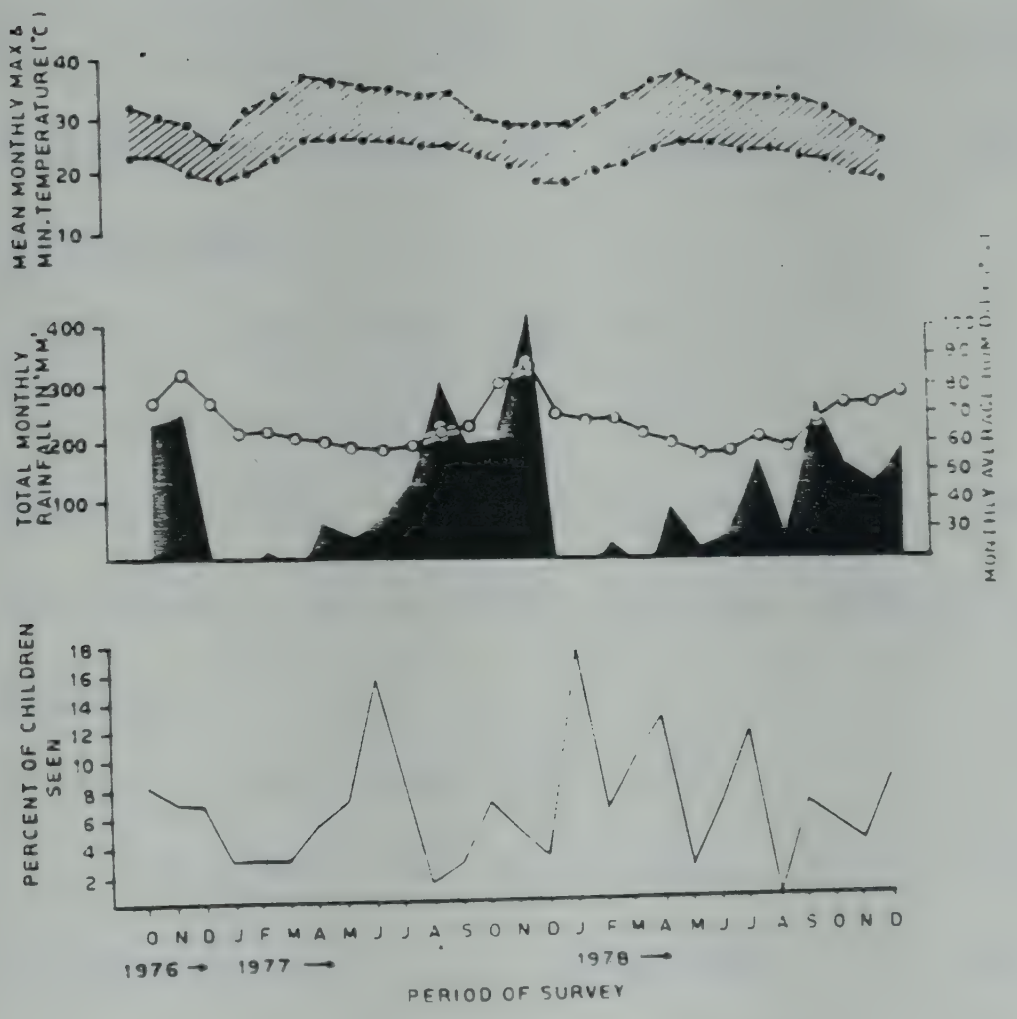


Fig. 3 CORRELATION OF CLIMATIC FACTORS WITH GROUP ABHS PYODERMA AMONG ORPHANAGE CHILDREN



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# CHAPTER 13

## ATTEMPTS TO DETECT POST STREPTOCOCCAL GLOMERULONEPHRITIS (PSGN)

Initially, in the pilot study conducted in Kaniyambadi, though no clinical cases of PSGN was suspected, routine testing of urine for albuminuria was done on 916 children; there were 28 with albuminuria. They were advised to bring 24 hours urine for total protein & Addis count; but their response and cooperation were too poor.

In the subsequent surveillance programme, 5991 urine were tested (Table 13.1) routinely for albuminuria and initially 2 AGN was suspected, though asymptomatic. These children were admitted to the hospital and detailed workup done and confirmed as PSGN. Both had active pyoderma caused by ABHS strains, T-1 and T-25 respectively, both recognised nephritogenic types. This suggested that streptococcal pyoderma, which is commoner than pharyngitis, might precipitate PSGN oftener in S.Indian children. Regular concerted attempts are to be done to detect such cases to come to a definite conclusion on case finding.

Both boys responded well to treatment with Benzyl benzathine penicillin 1,200,000 units IM initially.

Later 2 more cases, one in RS05 and another in RS16 were picked up.

All were put on 3 weekly prophylaxis & followed up till April 1979.

Thus a total of 4 cases of PSGN were detected, 2 in the high school RS05 and 1 each in RS02 & RS16. All were seen at the first survey as normals, initially. Two were picked up on routine urine testing and subsequent laboratory investigations. Two others developed PSGN during the course of surveillance within 9 months to 2 years interval. Tests done to confirm initially are given in Table 13.1.

In addition, one more child was confirmed as PSGN in 1978 giving a total of 4 PSGN among rural school children giving an incidence of 1.028/1000, just with the simple urine examination. All cases were initially treated as PSGN and were put on 3 weekly Inj.Benzyl benzathine penicillin from the time confirmed till Apr.1979. A total of 11 to 45 injections were givden.

Examination of all, for microscopic hematuria could not be done, which findings would have been more contributory. Many suspected of albuminuria refused further tests and dropped out. None of the nearly 313 preschool children examined proved to have AGN, though 10 had albuminuria.

It was not our intention to concentrate on PSGN detection and surveillance and the PSGN case finding was the result of basic, simple urinalysis. If concerted attempts at PSGN detection was done, more cases would have come to light. Thus it is seen that even with basic, initial attempts incidence of PSGN seems to be high in this rural children. This finding is consistent with our finding of very high prevalence & incidence of streptococcal pyoderma

and admittedly most of the PSGN we encounter may be from nephritogenic strains of pyoderma cases. A closer and intense programme of PSGN detection and surveillance is indicated in rural areas with high incidence of streptococcal pyoderma.

TABLE 13.1

DATA ON PSGN IN FIFTEEN SCHOOLS;

1ST, 2ND & 3RD SURVEY OF 10 SCHOOLS AND 1ST, 2ND

SURVEY OF 5 SCHOOLS

| DATA | RESULTS | |
|---|---|---|
| | 1975-76 | 1977 |
| NO. URINE TESTED | 5491 | 8670 |
| ALBUMIN POSITIVE | 27 | 75 |
| MICROSCOPY DONE | 26 | 391 |
| SIGNIFICANT MICROSCOPY | 7 | 11 |
| 24 HOURS ALBUMIN DONE | 7 | 1 |
| ADDIS COUNT ( 12 HOURS) | 9 | 1 |
| CONFIRMED AS PSGN TILL 1977 | 2 | 1 |

# CHAPTER 14

## SUMMARY, IMPACT & ACHIEVEMENTS OF STREPTOCOCCAL SURVEILLANCE PROGRAMME IN RURAL SOUTH INDIAN SCHOOL CHILDREN

During the last two decades, much progress has been made with regard to the advancement in the knowledge of bacteriology, serology/immunology, pathogenetic mechanisms and diagnosis of rheumatic fever (RF), rheumatic heart disease (RHD) and also of post streptococcal glomerulonephritis (PSGN) and their preceeding streptococcal infections. Much interest has been taken by World Health Organization and other such bodies in activating the interest of international bodies geared towards knowledge on the etiologic organism and the sequelae and their prevention. Much has been achieved in the affluent countries in bringing down the prevalence and incidence particularly of RF & RHD. The activities of W.H.O. and other bodies geared to an impact on lowering the incidence of RF/RHD in the developing countries of South East Asia, Mediteranean countries, Africa and South America is slowly drawing dividends. Whereas research & concentrated attempts to reduce the incidence was minimal before 1970s, the National bodies in India have been sensitised to the problem and much interest and co-operation at International level is witnessed from the 1970s onwards.

It was such an attempt of the W.H.O. which stimulated the chief investigator to start a Pilot programme on

surveillance of streptococcal infection & sequelae in a rural community in South India as early as 1973. A lot of information were gathered by utilising field workers, social workers and school teachers in getting preliminary data from 23 villages and their village schools in that Pilot programme. The prevalence of streptococcal pharyngitis was found to be low (4.24%) and streptococcal pyoderma very high (14.94%) while RF & RHD prevalence was 5.4/1000 among those rural school children.

Soon it was realised that, the task in this large rural community was formidable with limited resources & personnel and that it was better to restrict any further surveillance to the rural school children alone. It was at this time the Netherlands Ministry of Development and Co-operation supported this long term streptococcal surveillance programme from 1975 which was wound up by December 1978.

What were the achievements? What was the impact of the programme on the people in this area? How has this contributed to advancement in knowledge and development and what was its impact in India on the National front?

Summary and Impact of surveillance in the community

After updating the demographical data of the 13 villages in the Kaniyambadi block and all the 15 schools, a total of 3890 school children from 5 to 15 years were enrolled in the study. The male to female ratio was 3:2. In addition, data on 2015 children, of whom 1281 in the 0-5 year age-group of pre-school children & 734 of non-school

goers in the 5-15 years were also surveyed for a limited period (Chapter 5).

The normal carrier rate of beta-haemolytic streptococci (BHS) in the different seasons, during the first year of study was established as 17.2%, 5.1% and 4.9% in the throat, skin & nose respectively. The higher carrier rate was in winter and accounted for 33.2% BHS in the throat, with 8.2% ABHS, 2.1% in summer and least (1.6%) in the rainy season. Skin carriage was 15.1% BHS in winter, of which 12.9% was ABHS. Nasal carrier rate of ABHS in winter was 4.3%. As in our previous studies, a high throat carrier rate of group C and G was seen in school children.

The prevalence of streptococcal pharyngitis was assessed on bacteriology alone, as collection of blood for serology was often difficult. Admitting that with the best laboratory techniques employed, some of the BHS and ABHS isolated might have included some carriers also, the prevalence was still as low as 0.9%. It must be noted that in 23 children from whom blood was obtained for serology, 91.3% showed elevated ADNB titre (Chapter 7).

Unlike in pharyngitis, there was a high prevalence of streptococcal pyoderma in the same population of school children and was 9.4%; of these 7.7% was ABHS and the rest non-A. Pyoderma was more common in the 6-10 age-group and more, in boys.

Inspite of an active surveillance programme, and prophylaxis, the pyoderma was regularly found in different

schools during each subsequent surveys, showing that it was endemic among this population. It was a herculean task to eradicate pyoderma, unless the socio-economic conditions and indirectly the prevention of overcrowding, close contact & hygienic habits were improved among them. Moreover, the prolonged hot & humid climate, insect bites and injured skin getting contaminated with streptococci, was a greater challenge and these factors helped to perpetuate the problem of pyoderma in this community.

The prevalence of RF was established as 4.9/1000 in this rural school population. The greatest impact of the programme was lowering of the incidence of RF and RHD and fully controlling the incidence of the disease to a zero level (Table 13.1 & Fig. 13.1). It was thus clearly demonstrated, in a rural South Indian community of school children, that RF/RHD could be prevented.

Achievements

1. Encouragement of Health Education in a Rural community:

As medical personnel were minimal on the project, the help of staff of the Community Health Department, field workers, health workers, social workers, nurses, auxilliaries, etc., were utilised in the project in educating the children, parents & teachers. On a regular basis, we arranged to give health talks, demonstration flash cards and stories on clean habits, better nutrition, cleanliness of the environment and how to get medical

attention at times of illness so that further complication can be prevented. The children, parents & teachers were educated on how to take care of streptococcal infections & how important it is to take regular injection of long acting penicillin, both as a treatment of the current infection and as a primary prophylaxis to prevent sequelae.

In general, the surveillance programme had an impact on the general health & habits of the children and adults in the community. Thanks to the emphasis by the Department of Community Medicine on health, continued health education, cleanliness, prevention & better health care & nutrition of children and adults in the Kaniyambadi block.

Education of children & parents along with the teachers had much impact on:

a) Decline of absentism (Maxium 12%)

b) Understanding of streptococcal infection problem

c) Prevention of RF & RHD

d) Increase in compliance of primary & secondary prophylaxis

2. Long term Achievement:

In a later study in Kaniyambadi[1] block on the prevalence of various heart disease in the above community, by the Dept. of Commmunity Medicine, it was found out that the prevalence rate of RHD was 2.9/1000. Out of 17 RHD cases, 88% were school goers; 82% of RHD had a compliance rate of more than 75%. These findings certainly reflected the long term impact of the surveillance programme on the

same community in subsequent years.

It is possible that side by side with decline in RF incidence in this community by continuous monitoring, a reduction in the occurence of PSGN was also an indirect effect, as high compliance at primary & secondary prophylaxis of streptococcal infection was achieved by the programme.

It must be remembered, that despite the high compliance in pyoderma, the incidence of pyoderma did not alter much as already discussed above.

3. Improvements in Laboratory Technology:

Many modifications of older techniques & development of newer ones were carried out, viz:

a) Sand Dessication for preservation of Streptococci & other bacteria[2]

b) Improvements in filter paper technique with subsequent streaking of original site after 4 hours' incubation[3]

c) Higher isolation of streptococci by gentian violet blood agar for skin swabs

d) Introduction of gel precipitation test in addition to Lancefield technique for grouping & typing streptococci

e) Introduction of coagglutination test as a 3rd method of grouping which was found to be rapid, reliable, specific & cost effective[4,5]

f) CAMP test for detection of group B streptococci

g) Modification of antihyaluronidase test into a microtiter test

h) Introduction of finger-prick technique for blood collection for serology[6]

i) Production of locally non-available streptococcal sera

i) all grouping sera

ii) all T-typing sera

iii) common M-type antisera (12 types)

j) Introduction of ADNB test for the first time with Dr. J. Rotta, W.H.O. consultant's help

k) Production of reagents indigenously

Streptolysin 'O'

DNase B enzyme production & fractionation

DNase B substrate

DNase B control sera

Coagglutination reagent for grouping

l) NaDase, detection and typing

m) Serum opacity factor testing using Ox sera

4. Achievements in Continuing Education & Training:

i) Recognition of the Streptococcal Laboratory at Vellore by the Indian Council of Medical Research (ICMR) and institution of a "Streptococcal Reference Laboratory for Southern India" in 1980.

ii) Training of Laboratory workers from various Medical Colleges in India, including technicians and

scientific staff for exposure to various techniques in Streptococcal bacteriology and serology.

iii) Supply of antisera, strains, reagents, biologicals and literature for Streptococcal work to various scientific and technical staff in India.

iv) Institution of research activities by three post-graduate students leading to their Ph.D. Degrees.

v) Pulbication of important findings of our work in journals of national and international repute.

vi) Funding of another streptococcal research project by Department of Science & Technology, Government of India, on "Alternate approaches for the prevention & Management of RF & RHD" from 1982 to 1987.

vii) ICMR multicentric study on "Development of a community module for the prevention and management of RF and RHD" - Vellore being one of the 3 centres in India selected to study and develop a community module for the prevention of RF/RHD, on a national level.

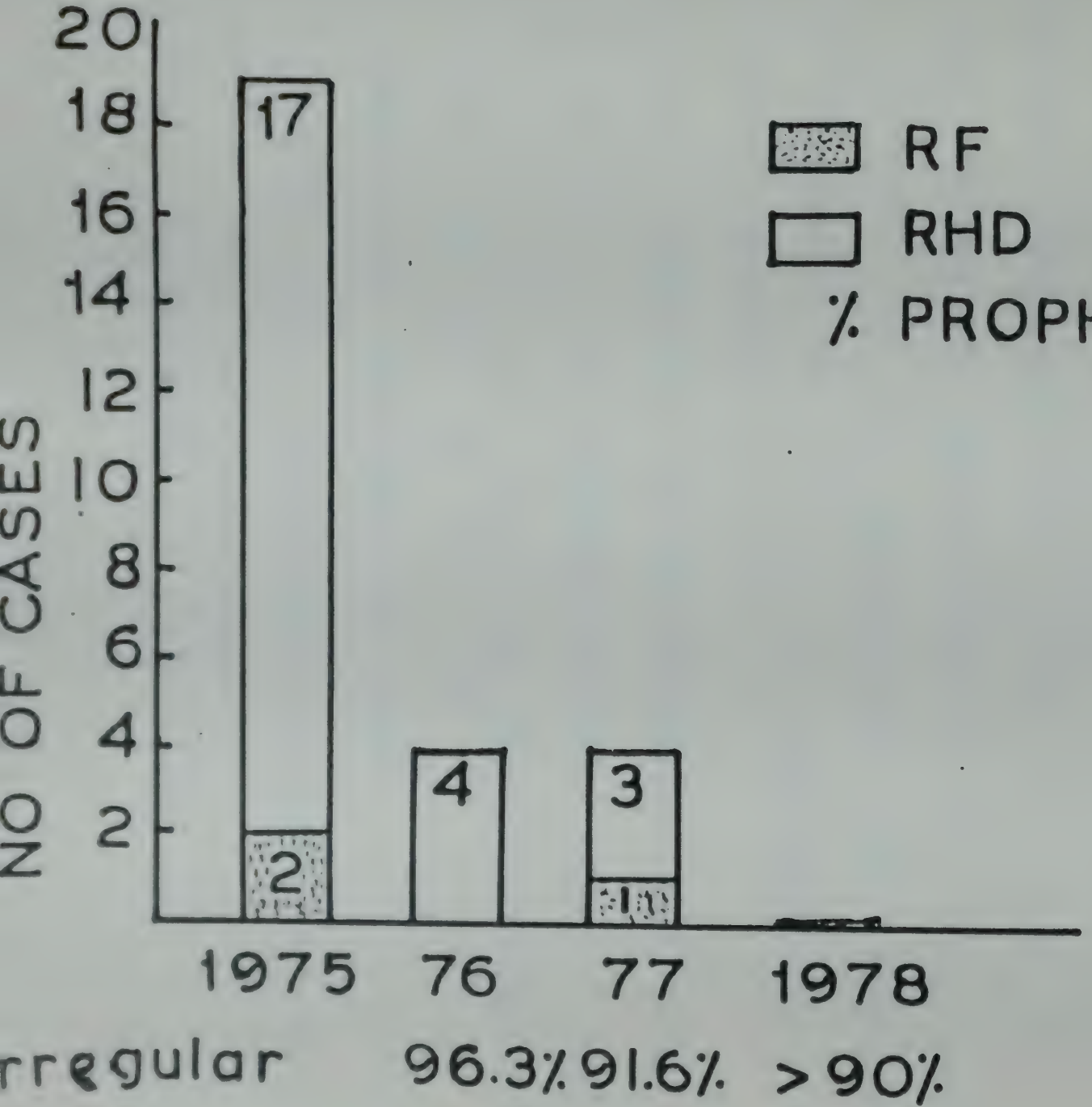
viii) Introduction of Lyophilization of all streptococcal grouping & typing strains, some sera, ASO and ADNB standards & other biologicals. This was made possible by the generous gift of the Lyophilizer by the Netherlands Government.

TABLE 14.1

IMPACT OF HEALTH EDUCATION

| DISEASE | COMPLIANCE AT PROPHYLAXIS | |
|---|---|---|
| | PRIMARY | SECONDARY |
| PHARYNGITIS | 100% (From 4th Survey) | |
| PYODERMA | 90% (From 4th Survey) | |
| RF, RHD | | 1975 - IRREGULAR<br>1976 - 96.3%<br>1977 - 91.6%<br>1978 - 90.0% |

Fig. I. IMPACT OF SURVEILLENCE PROGRAMME ON CONTROL OF RF, RHD AMONG 3890 RURAL SCHOOL CHILDREN



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## CONCLUSION

Rheumatic fever & Rheumatic heart disease continue to be a significant cause of high mortality & morbidity in the developing countries despite the fact that it is perhaps the most preventable of cardiac diseases and that the methods of their prevention are known and have been shown to be cost-effective. The study, reported herein, has been one of the earliest and well controlled surveillance study, in South India. With the concerted efforts of a collaborative team, it has been possible to achieve the goals, advocated by many world bodies in preventing this crippling and killing disease.

In a continuously done surveillance of streptococcal infections and sequelae among school children of 15 rural schools in Kaniyambadi block near Vellore with a strength of 3890 members surveyed at any time, the normal carrier rate in the throat, skin and nose in different seasons was established; maximum carrier rate was in winter or cold season in the throat (33.2%) followed by the skin (15.1%). The percentage of large number of group A (8.2%) carried on the skin was much higher than non-group A, unlike in other sites.

Streptococcal pharyngitis prevalence was very low (0.9%) in this communnity while streptococcal pyoderma was highly prevalent and accounted for 7.7%. The incidence of the already low pharyngitis rate declined to almost zero

level in some schools during surveillance, due to very high prophylactice coverage. The picture of pyoderma was entirely different, inspite of proper primary prophylaxis. The incidence continued between 5.9% and 9.8% in different surveys with a mean of 7.3%.

The compliance at primary prophylaxis to treatment of pharyngitis & pyoderma increased with better health education. As the rapport of the health team with the children, parents & teachers increased, their awareness and attitudes to the programme changed for the better.

The prevalence of RF & RHD in these children was found to be 4.9/1000 in 1975. With health education & increase in the compliance to prophylaxis, we were able to reduce the incidence to 1.7/1000 in 1976, to 1.6/1000 in 1977 & nil in 1978.

Thus, by this long term surveillance in a South Indian community, we were able to demonstrate that primary & secondary prophylaxis was not only feasible but very successful & accepted by the rural community, to an extent that could be expected in any country. The I.C.M.R. and other policy making bodies in India has taken note of this and along with other studies in Delhi, has initiated multicentric studies in India aimed at introducing the feasibility of similar control programmes in different states at the level of districts and primary health centres.

This chief investigator will be more than rewarded for her long years of work on streptococcal diseases and

prevention of RF & RHD, if only the workable module being tried out at different centres in India are really put into practical use in all districts in India before 1988. The collaborators and all the team workers will be more than happy if their understanding, collaboration & hard work in the field, hospital & laboratory during long months of hot & dry season, come to practical application before long, and the maxim "Health for All by 2000 A.D." becomes a reality in India.

Gratefully submitted,

DR. GRACE KOSHI,
Prof. & Head of Microbiology,
Principal Investigator,
Christian Medical College & Hospital,
VELLORE - 632 004, SOUTH INDIA.

# APPENDIX_I A_FAMILY CARD

ANCE OF STREP. INFECTIONS IN A S. INDIAN COMMUNITY MED. PROGRAMME H. H. NO. _____

F FAMILY

House No. & Street

Village.

Community | Veg: No / Yes | Average Annual Income of the Family

House

Mud / Tiled / Cemented
Thatti / Mud / Raw Brick / Burnt Brick / Plast / Cem. /
Thatched / Tiled / Terraced

Size : Sq. ft. (approx)
No. of rooms :
Electrified : Nil / Yes

outlet : Nil / Yes. If yes, Not satisfactory / Satisfactory
tion : Nil / Poor / Adequate

n

sh Disposal : Thrown indiscriminately / Heaped up / Stored in pits
ge : Nil / Open
: Nil / Public / Common / Seperate to HH
: Own open well / Common well / Tank / Public tap / House tap
seen : Nil / Yes. If yes, Flies Mosquitoes Others (Specify)
as H.M.S.)

ls & Pets : Buffalo Cattle Sheep / Goat Location : Inside same house/
Nos.) Pig Donkey Dog / Cat separately———ft. away
Fowl Duck Others

| Members in the House | Sex | Rel. to Head | Date of Birth Mo. Yr. | Martial | Education Sch. Col. | Occupation Prim. Second | Remarks |
|---|---|---|---|---|---|---|---|
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
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| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |

wer : Date

sor : Date

DEPARTMENT OF MICROBIOLOGY, C. M. C. HOSPITAL, VELLORE - 632004

73 P. T.O.

# APPENDIX - I A

## QUESTIONNAIRE FOR PRESENT* STREP DISEASES

| History & Symptoms (Yes/No) | Member Numbers | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 01 | 02 | 03 | 04 | 05 | 06 | 07 | 08 | 09 |
| Sore throat : | | | | | | | | | |
| Pain | | | | | | | | | |
| Cough | | | | | | | | | |
| Fever | | | | | | | | | |
| Skin infection | | | | | | | | | |
| Joint pains - specify | | | | | | | | | |
| Urine : scanty | | | | | | | | | |
| Blood in urine | | | | | | | | | |
| Difficuly in urin. | | | | | | | | | |
| Oedema of legs | | | | | | | | | |
| Dyspnoea | | | | | | | | | |
| Others | | | | | | | | | |
| Register No. (If registered) | | | | | | | | | |

Each member is to be questioned on each of the symptoms. Enter as " Yes " or " No " If any answe
give time of occurrence, duration, etc. A patient suspected of Streptococcal pharyngitis or pyoderma, RF
has to be registered accordingly and appropriate forms filled in. Refer to Medical Officer for detailed exa
of each suspect

Present and during the past one month

# APPENDIX _ IB

## MEMBER REGISTRY

Age Sex

Birth Date : Mo Yr

H. H. No. _____

Member No. _____

Pupil No. _____

P. P. No. _____

R. F. / AGN No. _____

Micro No. _____

E. C. G. No. _____

X-Ray No. _____

Date of 1st. Reg. _____

formation as shown below.

iceased, fill in appropriate cards.

Health : Poor / Fair Good

: Severe / Moderate / Mild / None

ition : Severe / Moderate / Mild / None

: Yes / No If yes, enter as present / past / recurrent

| ars \ Date | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| ealth | | | | | | | | | | |
| a | | | | | | | | | | |
| ition | | | | | | | | | | |
| roat | | | | | | | | | | |
| ma | | | | | | | | | | |
| ection | | | | | | | | | | |
| | | | | | | | | | | |
| ains | | | | | | | | | | |
| site | | | | | | | | | | |
| omplaints | | | | | | | | | | |
| essness | | | | | | | | | | |
| infn. in r members | | | | | | | | | | |
| infn. in bours | | | | | | | | | | |
| ewer | Sig. | | | | | | | | | |
| sor | & | | | | | | | | | |
| fficer | Date | | | | | | | | | |

# APPENDIX - IB

| Particulars \ Date | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
|---|---|---|---|---|---|---|---|---|---|
| Gen. Health | | | | | | | | | |
| Anaemia | | | | | | | | | |
| Malnutrition | | | | | | | | | |
| Sore Throat | | | | | | | | | |
| Pyoderma | | | | | | | | | |
| Ear infection | | | | | | | | | |
| Fever | | | | | | | | | |
| Joint pains | | | | | | | | | |
| Edema-site | | | | | | | | | |
| Urin. complaints | | | | | | | | | |
| Breathlessness | | | | | | | | | |
| Similar Infn. In other members | | | | | | | | | |
| Similar Infn. In neighbours | | | | | | | | | |
| Interviewer / Supervisor / Med. Officer | Sig. & Date | | | | | | | | |

# APPENDIX_IC

## PHARYNGITIS

H. H. No. _____

MEMBER No. _____

PUPIL No. _____

P. P. No. _____ RF No. _____

MICRO. No. _____ AGN No. _____

E _____ Age _____ Birth _____ Mo. _____ Yr. _____
Sex Date

e Code/School Code

| y No./Date | | | | | |
|---|---|---|---|---|---|
| al Health Fair/Good | | | | | |

| RYNGITIS - HISTORY | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| throat | Yes/No | Dura-tion | Yes/No | Dura-tion | Yes/No | Dura-tion | Yes/Nc | Dura-tion | Yes/No | Dura-tion |
| rent | Yes/No | | Yes/No | | Yes/No | | Yes/No | | Yes/No | |
| | Yes/No | | Yes/Nc | | Yes/No | | Yes/No | | Yes/No | |
| ing | Yes/No | | Yes/No | | Yes/No | | Yes/No | | Yes/No | |
| hroat | YesINo | | Yes/No | | Yes/No | | Yes/No | | Yes/No | |
| | Yes/No | | Yes/No | | Yes/No | | Yes/No | | Yes/No | |
| is | Yes/No | | Yes/No | | Yes/No | | Yes/No | | Yes/No | |
| fection | Yes/No | | Yes/No | | Yes/No | | Yes/No | | Yes/No | |
| rent | Yes/No | | Yes/No | | Yes/No | | Yes/No | | Yes/No | |
| s: PYODERMA | Yes/No | | Yes/No | | Yes/No | | Yes/No | | Yes/No | |
| | | | | | | | | | | |

### PHARYNGITIS — CLINICAL DATA

| y No. Date | | | | | | Survey No. Date | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| s | N/E/A | N/E/A | N/E/A | N/E/A | N E/A | Congestion of Pharynx | Yes/No | Yes/No | Yes/No | Yes/No | Yes/No |
| stion | Yes/No | Yes/No | Yes/No | Yes/No | Yes/No | | | | | | |
| | Yes/No | Yes/No | Yes/No | Yes/No | Yes/No | Cer. lymph adenitis | Yes/No | Yes/No | Yes/No | Yes/No | Yes/No |
| e | Yes/No | Yes/No | Yes/No | YesINo | YesINo | Temp. (P.A.) | | | | | |
| es | Yes/No | Yes/No | Yes/No | Yes/No | Yes/No | | | | | | |
| | | | | | | Qthers | | | | | |

| of Follow up Findings | | | | | |
|---|---|---|---|---|---|
| | | | | | |
| nfection | | | | | |
| | | | | | |
| Pains | | | | | |
| tion | | | | | |
| r | | | | | |
| | | | | | |
| y Complaints | | | | | |
| | | | | | |
| | | | | | |

# APPENDIX - Ic

## LABORATORY DATA

| SURVEY No. / DATE | | Foll. up | | Foll. up | | Foll. up | | Foll. up | |
|---|---|---|---|---|---|---|---|---|---|
| **Culture** (Strep Group & type) Throat | | | | | | | | | |
| Nose | | | | | | | | | |
| Skin | | | | | | | | | |
| Ear | | | | | | | | | |
| **Serology** ASO | | | | | | | | | |
| AH | | | | | | | | | |
| ADNB | | | | | | | | | |
| C-RP | | | | | | | | | |
| **Urine** Albumin | | | | | | | | | |
| Sugar | | | | | | | | | |
| WBC | | | | | | | | | |
| RBC | | | | | | | | | |
| Casts | | | | | | | | | |
| Others | | | | | | | | | |
| | | | | | | | | | |

## TREATMENT

| | | | | | |
|---|---|---|---|---|---|
| Penidure (Enter dates given) Local Treatment | | | | | |
| Date & Signature of Medical Officer | | | | | |

# APPENDIX_ID

## PYODERMA

H. H. No.

MEMBER No.

PUPIL No.

P. P. No. RF No.

MICRO. No. AGN No.

Age Sex Birth Date Mo. Yr.

ode/School Code

| | | | | | |
|---|---|---|---|---|---|
| /Date | | | | | |
| ealth Good | | | | | |

| MA - HISTORY ction | Yes/No | Dura-tion | Yes/No | Dura-tion | Yes/No | Dura-tion | Yes/No | Dura-tion | Yes/No | Dura-tion |
|---|---|---|---|---|---|---|---|---|---|---|
| | Yes/No | | Yes/No | | Yes/No | | Yes/No | | Yes/No | |
| | Yes/No | | Yes/No | | Yes/No | | Yes/No | | Yes/No | |
| omplaints | Yes/No | | Yes/No | | Yes/No | | Yes/No | | Yes/No | |
| URI | YesINo | | Yes/No | | Yes/No | | Yes/No | | Yes/No | |
| | | | | | | | | | | |

## PYODERMA — CLINICAL DATA

| . AND DATE KIN LESION: | | | | | | SURVEY No. AND DATE / SITE OF SKIN LESION : | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Scalp | | | | | |
| | | | | | | Face | | | | | |
| | | | | | | Ears | | | | | |
| is | | | | | | Neck | | | | | |
| | | | | | | Chest | | | | | |
| | | | | | | Back | | | | | |
| | | | | | | Axilla | | | | | |
| a | | | | | | Arms | | | | | |
| rickly heat | | | | | | Fingers : Webs | | | | | |
| | | | | | | Nail bed | | | | | |
| ea | | | | | | Groin | | | | | |
| | | | | | | Buttocks | | | | | |
| is | | | | | | Thigh | | | | | |
| | | | | | | Legs | | | | | |
| | | | | | | Feet | | | | | |
| nfection members uration | | | | | | Others | | | | | |
| nfection ours uration | | | | | | | | | | | |
| en | | | | | | Sign of Medical Officer & Date | | | | | |

SURVEILLANCE OF STREPTOCOCCAL INFECTIONS, C. M. C. HOSPITAL, VELLORE F. T O.

# APPENDIX -ID

## LABORATORY DATA

| SURVEY No. / DATE | | Foll. up | | Foll. up | | Foll. up | | Foll. up | |
|---|---|---|---|---|---|---|---|---|---|
| **Culture** (Strep Group & type) Throat | | | | | | | | | |
| Nose | | | | | | | | | |
| Skin | | | | | | | | | |
| **Serology** ASO | | | | | | | | | |
| AH | | | | | | | | | |
| ADNB | | | | | | | | | |
| C-RP | | | | | | | | | |
| **Urine** Albumin | | | | | | | | | |
| Sugar | | | | | | | | | |
| WBC | | | | | | | | | |
| RBC | | | | | | | | | |
| Casts | | | | | | | | | |
| Others | | | | | | | | | |
| Dates of Follow up and Findings | | | | | | | | | |
| Lesion healed or not | | | | | | | | | |
| New Infection | | | | | | | | | |
| Fever | | | | | | | | | |
| Joint Pains | | | | | | | | | |
| Palpitation | | | | | | | | | |
| Murmur | | | | | | | | | |
| Edema | | | | | | | | | |
| Urinary Compl. | | | | | | | | | |
| Others | | | | | | | | | |
| | | | | | | | | | |

## TREATMENT

| | | | | | |
|---|---|---|---|---|---|
| Penidure (Enter dates given) Local Treatment | | | | | |
| Date & Signature of Medical Officer | | | | | |

# APPENDIX_IE

## RHEUMATIC FEVER (RF) REGISTRY

H. H. No.

| | | | |
|---|---|---|---|
| | Age Birth : Mo. Yr. | Member No. | RF Reg. No. |
| s : | Sex Date | Hosp. No. | PP No. |
| l Health : Poor / Fair / Good | | E. C. G. | Micro. No. |
| | | X-ray | Date of Regn. |

| y of RF | | | If Yes, Duration Days | Mo. | Yr. | Not known | | | | If Yes, Duration Days | Mo. | Yr. | Not known |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| dant sore throat | No | Yes | | | | | Recurrent | No | Yes | | | | |
| dent Skin Infect. | No | Yes | | | | | Recurrent | No | Yes | | | | |
| | No | Yes | | | | | Recurrent | No | Yes | | | | |
| ains | No | Yes | | | | | Recurrent | No | Yes | | | | |
| igratory | No | Yes | | | | | Recurrent | No | Yes | | | | |
| a | No | Yes | | | | | Recurrent | No | Yes | | | | |
| pain | No | Yes | | | | | Recurrent | No | Yes | | | | |
| lessness | No | Yes | | | | | Degree of breathlessness : rest/mod/severe / noct | | | | | | |

of RF : Mo. Yr. If recurrent, give dates

| | | | |
|---|---|---|---|
| in other members | No/Yes/Duration | Rel. ship | Member No. |
| throat : in other members : | No/Yes/Duration | Rel ship | Member No. |
| nfect : in other members : | No/Yes/Duration | Rel. ship | Member No. |
| Type of lesion | | | |

ICAL DATA WHEN FIRST SEEN

. (P.A.) Pulse : B.P. :
Weight : Heart rate :
t : Tonsils : Normal/Enlarged/Absent
Redness, Oedema
Follicles, Exudate
Submandibular gland : Swollen, tender
lesions : No/Yes, If Yes, site
Type of lesion
s : Tender, swollen, hot, red. movements limited
Migratory or not
Joints effected

Erythema marginatum : No/Yes/Doubtful
Subcutaneous Nodules : No/Yes/Doubtful
Chorea No/Yes/Doubtful
Murmurs : Mitral, Syst, Diast,
Aortic, Syst, Diast,
Cong. failure : No/Yes (Oedema, ascitis dyspnoea, oliguria)
Fever of long duration (S. B. E.)
Nephritis : No/Yes (puffy face, oliguria, dysuria, haematuria,) Urinary infection.
Other diseases : No/Yes

VISIONAL DIAGNOSIS ;

DIOLOGIST'S NOTES :

| L DIAGNOSIS | Definite Carditis : No/Yes | Suspect Carditis : No/Yes | Other conditions diagnosed (related or unrelated) |
|---|---|---|---|
| e Rh. Fever | | | 1 |
| l stenosis | | | 2 |
| c stenosis | | | 3 |
| l insufficiency | | | 4 |
| c insufficiency | | | 5 |
| Heart failure | | | |

E : All informations on this page are to be filled in when first seen.

SURVEILLANCE OF STREPTOCOCCAL INFECTIONS. C. M. C. HOSPITAL, VELLORE

P. T. O.

76

# APPENDIX - IE

## MONTHLY FOLLOW UP RECORD - RF PROPHYIAXIS

| Enter Dates seen | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| HISTORY ; If Yes, duration | | | | | | | | | | | |
| U. R. I. No Yes | | | | | | | | | | | |
| Pyoderma No Yes | | | | | | | | | | | |
| Other infns. No Yes | | | | | | | | | | | |
| Joint pain No Yes | | | | | | | | | | | |
| If Yes, site | | | | | | | | | | | |
| Chorea No Yes | | | | | | | | | | | |
| Palpitation No Yes | | | | | | | | | | | |
| CLINICAL FINDINGS : Pharyngitis | | | | | | | | | | | |
| Tonsiles | | | | | | | | | | | |
| Skin infection-site | | | | | | | | | | | |
| Arthritis-site | | | | | | | | | | | |
| Arthralgia-site | | | | | | | | | | | |
| Erythema marginatum - Site | | | | | | | | | | | |
| Subcutaneous Nodules - Site | | | | | | | | | | | |
| Chorea | | | | | | | | | | | |
| Temperature | | | | | | | | | | | |
| Heart rate | | | | | | | | | | | |
| B.P. | | | | | | | | | | | |
| PROVISIONAL DIAGNOSIS | | | | | | | | | | | |

| LAB. DATA | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Throat / Skin / Nose — Beta-strep. Group/Type Nil | | | | | | | | | | | |
| ASO | | | | | | | | | | | |
| ADNB | | | | | | | | | | | |
| AH | | | | | | | | | | | |
| C-RP | | | | | | | | | | | |
| PCV/Hb. | | | | | | | | | | | |
| ESR | | | | | | | | | | | |
| WBC | | | | | | | | | | | |
| URINE : Alb. | | | | | | | | | | | |
| Sugar | | | | | | | | | | | |
| Micro. | | | | | | | | | | | |
| E. C. G. | | | | | | | | | | | |
| X-ray | | | | | | | | | | | |
| OTHERS : | | | | | | | | | | | |
| TREATMENT & PROPHYLAXIS Dates Injections taken: | | | | | | | | | | | |
| CARDIOLOGISTS' Seen : Yes / No Notes, Date | | | | | | | | | | | |
| Patient : Moved, Not traced | | | | | | | | | | | |
| Medl. Officer Sig. / Date : | | | | | | | | | | | |
| Interviewer : Date | | | | | | | | | | | |
| Supervisor : Date | | | | | | | | | | | |

Note :—Refer to Dr. George Cherian when any acute rheumatic fever or rheumatic heart diseases is susp for detailed examination.

# APPENDIX_IF

## CUTE GLOMERULONEPHRITIS — REGISTRY

H. H. No.

Age Birth Mo. Yr.
Sex Date

MEMBER NO.

REG. No. A-

Health : Poor/Fair/Good

PP No. (if any)

ECG No.

MICRO. No.

DATE OF REGN.

| Y / oat | No | Yes | If yes, duration Mo. | Yr. | Not known | Recurrent | No | Yes | If yes, duration Mo. | Yr. | Not known |
|---|---|---|---|---|---|---|---|---|---|---|---|
| a | No | Yes | | | | Recurrent | | | | | |
| | No | Yes | | | | Recurrent | | | | | |
| ria | No | Yes | | | | Recurrent | | | | | |
| | No | Yes | | | | Recurrent | | | | | |
| a | No | Yes | | | | Recurrent | | | | | |
| ion | No | Yes | | | | Recurrent | | | | | |
| | No | Yes | | | | Recurrent | | | | | |

in other members : No/Yes/Duration Rel. ship Member No.

oat : in other members : No/Yes/Dur. Rel. ship Member No.

in neighbours : No/Yes/Dur. Household No.

na : in other members : No/Yes/Type of lesion

Duration Rel. ship Member No.

in neighbours : No/Yes/Duration Member No. Hosehold No.

| CAL DATA | FIRST SEEN Date | Follow Up Date | Date | Date | Date |
|---|---|---|---|---|---|
| erature | | | | | |
| lesions | | | | | |
| of lesion | | | | | |
| of lesion | | | | | |
| yngitis | | | | | |
| ils (N/E/A) | | | | | |
| ma (+ —) | | | | | |
| ght | | | | | |
| | | | | | |
| pularus | | | | | |
| . failure | | | | | |

viewer Date Supervisor Date

APPENDIX - I F

| LABORATORY DATA | FIRST SEEN | Date of Follow Up and Findings | | | |
|---|---|---|---|---|---|
| Urine-Protein | gms/24 hrs | | | | |
| Sugar | | | | | |
| RBC | Many/Rare/None | | | | |
| WBC | Many/Rare/None | | | | |
| Gran. casts | Many/Rare/None | | | | |
| E. S. R. | | | | | |
| Blood urea | | | | | |
| Creatinine | | | | | |
| CR-P | | | | | |
| Others | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |

| BACTERIOLOGICAL/SEROLOGICAL DATA | Beta strep (HMS) | | Beta strep (HMS) | | Beta strep (HMS) | | Beta strep (HMS) | | C |
|---|---|---|---|---|---|---|---|---|---|
| | Group | Type | Group | Type | Group | Type | Group | Type | |
| Culture : Throat | | | | | | | | | |
| Nose | | | | | | | | | |
| Skin | | | | | | | | | |
| Ear | | | | | | | | | |
| Urine | | | | | | | | | |
| Antibody : ASO | | | | | | | | | |
| AH | | | | | | | | | |
| Others | | | | | | | | | |
| Other Tests | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |

| TREATMENT | | | | | |
|---|---|---|---|---|---|
| Medical Officer | | | | | |

## APPENDIX II
## MEDIA

1. Blood Agar (BA)

Ingredients: Sterile defibrinated sheep blood - 50 ml

Trypticase soy agar (BBL)- melted- 100 ml

Preparation

Trypticase soy agar was rehydrated and sterilized by autoclaving as per the instructions of the manufacturer. Sterile defibrinated sheep blood was added to the sterile agar previously melted and cooled to 45-50 $^{o}$C. The agar and blood were mixed well and poured into sterile petri dishes.

2. Crystal violet blood agar (CVBA)

Ingredients: Trypticase soy blood agar base - 100 ml

Crystal violet 0.1% solution - 0.2 ml

Sheep blood - 5-7 ml

Preparation

Dissolve 1 gm of crystal violet in one litre of distilled water. From this solution, add 0.2 ml to melted trypticase soy agar base and autoclave at 121 $^{o}$C for 15 minutes. Cool to 45 to 50 $^{o}$C and add 5 to 7 ml of sheep blood. Mix and pour plates.

3. Todd-Hewitt broth (THB) (Commercial dehydrated medium)

Ingredients: Beef heart infusion from (solid) - 3.1 g

Neopeptone - 20.0 g

Dextrose - 2.0 g

Sodium chloride - 2.0 g

Disodium phosphate - 0.4 g

Distilled water - 1000 ml

Final pH 7.8 $\pm$ 0.2

Preparation

Dissolve the ingredients in distilled water and sterilise by autoclaving at 121°C for 15 minutes.

4. Robertson's Cooked Meat medium (RCM)

Ingredients: Beef infusion broth

Minced and dried meat

Preparation

Use lean beef. Remove fat and connective tissue before grinding. Mix meat and water. Cool, refrigerate over-night and skim off any remaining fat. Boil for 30 minutes. Filter the mixture through two layers of gauze and spread the meat particles out to dry.

The dried meat particles are distributed in 4x1/2"( 15x150 mm or 12x100 mm) test tubes to a height of 1.5 to 2.5 cms (1 part). The BIB is then added to get 3 to 4 parts (v/v) liquid per tube. The broth should be sufficient to immerse the meat particles and extend about 2 cm above the meat. The 12x100 mm test tubes (or 15x150 mm for anaerobes) may be screw-capped or plugged with cotton and sterilized by autoclaving at 121°C for 15 minutes. For the preservation of stock cultures, the plugs are removed and the tubes are closed with paraffin coated corks.

## APPENDIX III

## Bacteriological Techniques

1. Filter-paper(FP) kit for transportation of specimens 1,2

Preparation

Whatman No.1 filter paper strip, 1.5x4.5 cm, marked on one side with pencil, is covered on both sides by butter paper, 2.5x11.0 cm, folded slightly off the middle. A pencil mark is made on the opposite side of the FP. This kit is wrapped in aluminium foil in such a way that the closure of the aluminium foil is on the side of the paper strip without the pencil mark (Fig. III-A). The whole kit is sterilized in the autoclave at 121 $^{o}$C for 15 minutes.

Transport of specimens

The aluminium foil wrapping is carefully unfolded and the butter paper is lifted, taking care not to touch the filter paper stripinside. The cotton swab with the specimen is then scrubbed and rolled over the surface of the filter paper strip with light pressure so as to transfer all the material on the swab to the FP. The filter paper is allowed to remain uncovered for one or two minutes for drying. The FP is again covered with the butter paper and then the aluminium foil is refolded over the butter paper and the kit is closed. The specimen number is marked on one end of the aluminium wrapping with a ball point pen. This kit is then transported to the laboratory.

Culturing of FP specimens

a) Common method

The FP kit is unfolded and the inoculated filter paper strip is removed with sterile forceps and placed face down (with the pencil mark facing upwards) on one half of a BA plate. The BA is incubated for 4 hours at $35^{o}C \pm 1^{o}C$. The filter paper strip is then lifted with sterile forceps and placed on the other half of the same plate and the incubation continued overnight. Next morning the FP is discarded into a lotion jar and the reading is taken.

b) Alternate method for culture and isolation of colonies

After placing the FP on one side of the BA and incubating for 3 hrs to 4 hrs, it is transferred to a fresh place of the BA or discarded. The original site where the FP was placed is inoculated on half plate, to get isolation of colonies. Continue incubation overnight. Thus, in the same plate, isolated colonies can be obtained overnight with no need for subculture[3]. The FP ends may be cut with a sterile forceps and the whole procedure of placing FP, removing it and inoculating for isolated colonies may be done using only 1/2BA, if so desired.

Use

(1) The FP kit may be used for transporting clinical materials from a distant field study to the laboratory.

(2) The FP kit may be used to send strains to reference laboratories or receive strains, as the FP can preserve the streptococci in a viable condition for a few weeks and for 10 to 14 days even under high humidity[1].

(3) It is also used for storage of streptococci for brief periods.

2. Group identification of beta-hemolytic streptococci

1) Extraction of group specific carbohydrate by Lancefield's method

Requirements

N/5 HCl prepared in 0.85% saline

N/5, N/10 and N/20 HCl prepared in distilled water

N/5, N/10 and N/20 NaOH preparation in distilled water

0.04% metacresol purple. Dissolve 0.4 g of metacresol purple in 100 ml of absolute alcohol and dilute 1/10 in distilled water

Capillary tubes (0.2 mm wall thickness; 1.2-1.5 mm outside diameter x 90 mm)

Wooden boards with grooves filled with plasticine

Procedure of Hot-acid Extraction

Pure cultures of isolates were grown overnight in 30 ml THB and the cells were collected by centrifugation. Sufficient N/5 NCl prepared in saline was added to the

cell sediment to produce a salmon pink colour of the metacresol purple indicator. After thorough mixing, the acidified cell suspension was heated in a boiling water bath for 10 minutes with shaking in between. The cellular material was then removed by centrifugation and the supernatant was decanted into a clean test tube. The pH of this extract was then adjusted to neutrality by the addition of N/5 NaOH. Final adjustments were made by using N/10 or N/20 NaOH or HCl as needed. The extract was again centrifuged and the clear supernatant was transfered to a clean test-tube.

Grouping of BHS by capillary precipitation

A column of grouping antiserum about 1.5-2.0 cm in height was permitted to rise in the capillary tube. The excess serum was then wiped off from the end of the capillary with tissue paper. An equal volume of extract was then taken into the capillary tube taking care not to introduce any air bubble between the serum and the extract. The whole column was allowed to run to the middle of the tube. The outside of the capillary tube was inserted into plasticine to stand in an upright position.

Reactions were read within 1 to 5 minutes and rarely between 5-10 minutes. The tubes were held against a bright light source from a multipurpose daylight fluorescent lamp using a dark background. Reactions that occurred within the first few minutes and

resulting in a heavy precipitate were read as strong (S) and if the reaction developed with smaller particles slowly and was less turbid, it was recorded as moderate (M). When reactions failed to develop within the first 5 to 10 min. results were recorded as negative.

Grouping of BHS by agar-gel precipitation

A Gelman immunodiffusion kit (Catalogue No.51445, Gelman Instrument Company, Ann Arbor, Michigan, U.S.A.) was used for the gel-diffusion tests. The agar-gel was prepared with Noble Agar (Difco Laboratories, Detroit, U.S.A.) at a concentration of 1 per cent in 0.85% NaCl. The pH was adjustd for 7.0 to 7.2 using 0.1 N NaOH and sodium azide was added to a final concentration of 0.01%. After melting the agar in a boiling waterbath, it was transferred to a 56 $^{o}$C waterbath. New and cleaned microscopic slides (25mm x 75mm) were kept on special Gelman plastic frames.

Ten to 12 ml of warm melted agar was then poured onto the frame with slides, starting with the gap between the slide edge and the frame. After preparing the necessary number of slides , the levelling table was covered with a lid and the slides were allowed to set for about 20 to 30 minutes. Wells were cut on the slides using a Gelman Punch. The plugs of agar from the punched out areas were removed by a suction needle attached to a water aspirator.

The central wells were always charged with antisera against group A, B, C or G and the peripheral wells with the test extracts, using fine tipped Pasteur pipettes. Special care was taken not to allow any overflow of the reactants.

The frames were then kept in a steel 'frame-holder' and held in a humid chamber. The whole 'set' was then kept at +4 °C for 16 to 18 hrs. The readings were taken by holding the frames against a multipurpose daylight fluorescent lamp against a dark background. Precipitin bands were looked for (+) against A, B, C or G antiserum wells

Grouping of BHS by Co-agglutination of antibody coated protein A containing Staphylococci



Principle

This method makes use of the unique property of Staphylococcus aureus protein-A to bind to the Fc' portion of IgG molecules, leaving the 'Fab' portion free for the specific antigen binding. Usually broth cultures of streptococci are used as antigens, and positive results depend on their co-agglutination by the antibody coated staphylococcal cells when tested on a ring slide. (Fig III-B)

Preparation of Staphylococcal reagent

The Cowan I strain of Staphylococcus aureus which consistantly produces large amounts of protein-A is grown in Todd-Hewitt agar (THB + 1.5% agar) overnight

and the washed cells are formalinized (0.5% formaldehyde in 0.03M phosphate buffered saline). After further washings, the formalinized cells are heated in a waterbath at 56°C for 30 minutes to remove the foul odour. After still further washings, the cells are suspended to 10% in PBS with 0.1% sodium azide.

Sensitization of staphylococcal reagent

For sensitization, 0.1 ml of the group specific streptococcal antiserum is added to 1.0 ml of the 10% staphylococcal suspension, mixed and left at room temperature for 30 minutes. After washing once, the sensitized cells are resuspended to 1% in PBS with 0.1% sodium azide as a preservative and stored at 4°C.

Each reagent thus prepared is checked with broth cultures of streptococci belonging to homologous and heterologus groups. A proper reagent should co-agglutinate only with streptococcal cells of the homologus group and not with those of other groups.

Co-agglutination technique

a) Grow the streptococci to be tested in 1.0 ml of THB for 4 to 6 hrs. or overnight

b) Using ceramic ring glass slides or ordinary concavity slides, mix one drop each of the group specific staphylococcal reagent and one drop of the TH broth culture of the streptococci

c) Rotate the slide manually for 1 minute

d) Observe the mixture for evidence of agglutination

e) If none of the mixtures show any agglutination, continue mixing for another minute and read results

f) The group of the streptococcal isolate will be that of the group reagent which gives the strongest agglutination

3. Type Identification of Group A Beta-hemolytic streptococci (ABHS)

Type identification of group A beta-hemolytic streptococci (ABHS) is indispensable for epidemiological purposes. Knowledge on the type of ABHS circulating in the community, or in individuals with infection is important in understanding the etiology and pathogenesis of streptococcal disease. It is not mandatory to type ABHS in routine diagnostic procedures.

M-typing of ABHS

M-typing of ABHS is based on the principle that most strains of ABHS elaborate the specific M protein which is antigenic and precipitates with its specific antibody.

Generally mucoid and matt colonies are M-typable. Glossy colonies are difficult to M-type. M-typability of ABHS have varied from 20% to 50% depending on the colony form, clinical situation, method of cultivation, virulence, etc. M-typing sera are produced by only a few workers in the world and are not available commercially.

Procedure

1. Use the hot-acid extract prepared according to the Lancefield procedure
2. Use capillary tubes, 0.7-1.0 mm O.D., which have been cleaned with facial tissue and sterilized in an autoclave (Note: Dry heat sterilization destroys the capillarity of the tubes as do plugging with cotton and many types of paper. Whatman filter paper is satisfactory for capping the tubes in which the capillary tubes are sterilized).
3. Draw into the capillary tube, a 1.5 cm column of absorbed M-typing serum.
4. Clean capillary carefully with tissue paper to avoid carry over of serum to the extract.
5. Draw up the extract into the same capillary to an amount equal to the serum column. If an air bubble separates serum and extract, discard capillary and repeat.
6. Clean capillary carefully with tissue paper to remove any extract left on the outside of the capillary.
7. Tip the capillary gently to permit the serum extract column to move to the centre of the tube.
8. Place the capillary vertically, in the plasticine, so that the extract is below the serum and the lower miniscus of the column is above the palsticine.
9. Examine after 5-10 min. for precipitation with a bright light against a dark background. A few strong sera may give a light reaction during this time. However, this

immediate examination is to check for non-specific precipitates in serum of extracts, dirty tubes, etc.

10. Incubate the tubes at 37°C for 2 hours and re-examine briefly. A strong reaction may be recorded at this time.

11. Refrigerate overnight at 4 to 10°C and then take final readings. A positive reaction is evidenced by a precipitate, and is usually graded as follows:-

Weak: A faint precipitate throughout the column, just visible to the naked eye after 2 hours of incubation and settling to < 0.5 mm at the base of the column after overnight refrigeration

Moderate: Larger amount of precipitate settling to 0.5 to 1.5 m at the base of the column.

Strong: Precipitate settling to > 1.5 mm

Note: Weak reactions are considered unsatisfactory for type designation of the test culture.

T-typing of ABHS

The identification of T-antigen is based on the agglutination of ABHS by specific typing antisera. This system, i.e. Griffith's system, differentiates ABHS into many T-types. Many strains of ABHS, carry more than one T-antigen and hence they are grouped into 'T-patterns'.

T-typing was carried out by slight modification of techniques advocated by two reference laboratories.

Procedure:

1. Inoculate a pure culture of the test strain into 5 ml THB and incubate overnight at 30°C.

2. Centrifuge and decant supernatant into disinfectant, leaving the cell sediment and about 0.3 ml of fluid at the bottom.

3. Add two drops of a 5% trypsin solution and one drop of Universal indicator.

4. Adjust to pH 8.0 with N/5 NaOH. (i.e. until the indicator turns greenish in colour. The pH usually comes down on standing, and hence it is better to add a slight excess of alkali, at the initial step.

5. Mix thoroughly on a vortex mixer and incubate at 37 $^{o}$C for 1 hour.

6. Bring down pH to 7.0 with N/5 HCl.

7. Using a 3 mm loop, place loopful of the cell suspension on a clean microscope slide (Five separate loopfuls can be readily placed on a single microscope slide). With a sterile loop, transfer a loopful of polyvalent serum and mix with the suspension

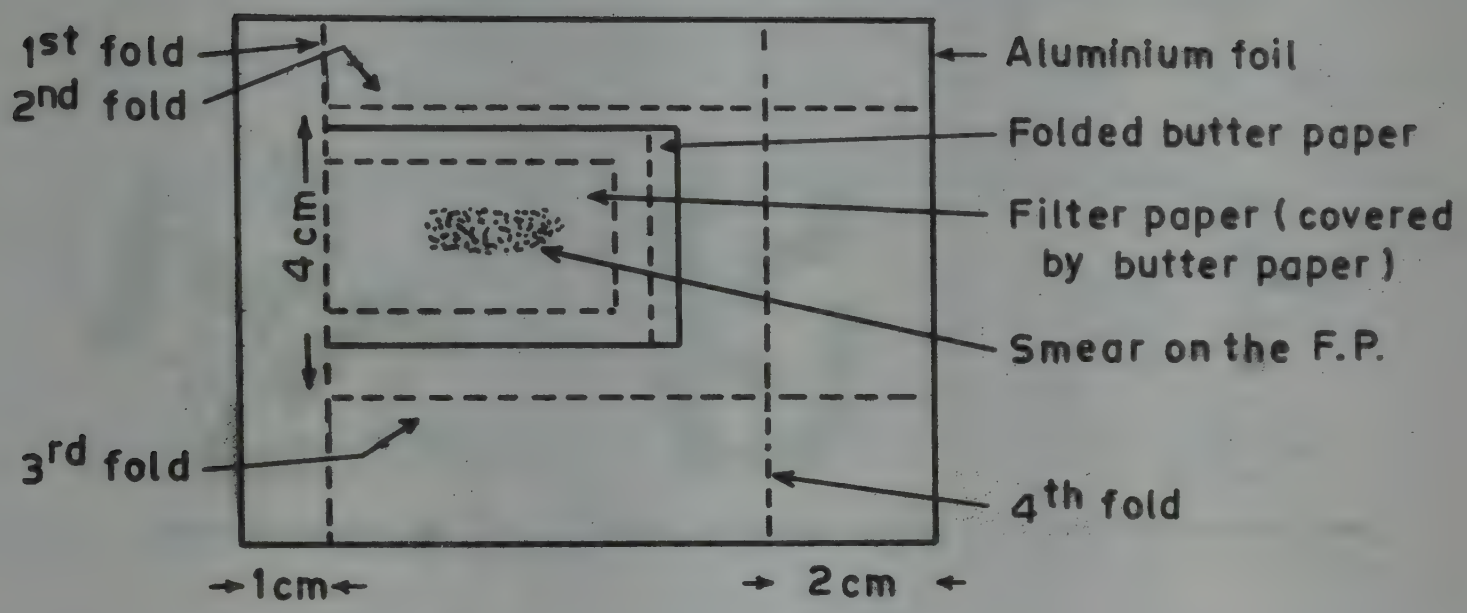
8. Rock the slide a few times and look for agglutination with the aid of a multipurpose daylight fluorescent lamp. Positive agglutination should become obvious within a few seconds or at least within a minute.

9. Test with monovalent sera of the particular pool serum. Record reaction as follows:-

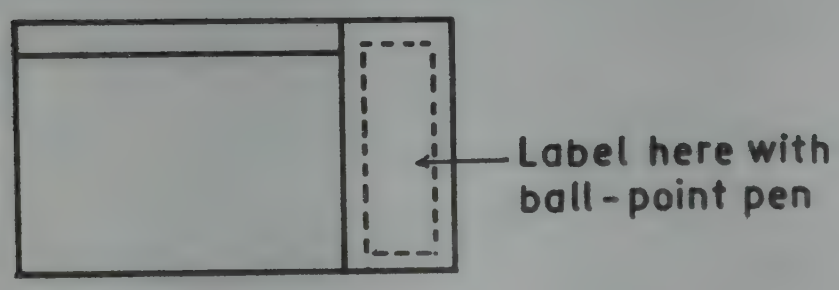
| | | |
|---|---|---|
| Weak but clear agglutination in 2-3 min. | - | + |
| Strong agglutination in 1 min. | - | ++ |
| Immediate strong agglutination with clearing | - | +++ |

10. If the suspension is granular, or homogenous but agglutinates with multiple pools, a second (at 37°C for 1 hour) or sometimes a third (at 50°C for 15-20 min.) trypsinization is carried out, till clear cut and specific reactions occur.

FIG III-A




The opened F.P. Kit




Folded F.P. Kit

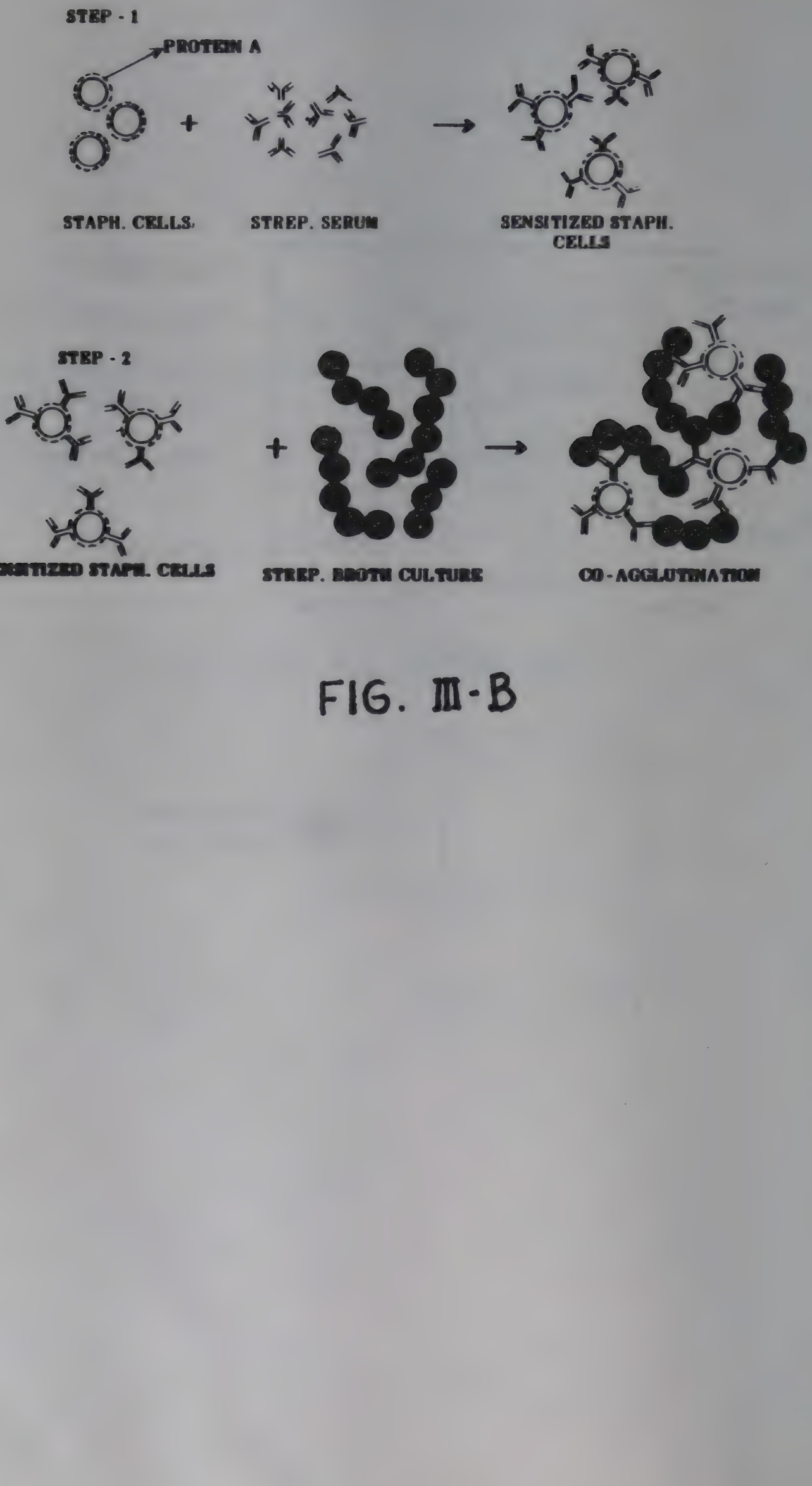

STEP - 1
PROTEIN A
STAPH. CELLS
STREP. SERUM
SENSITIZED STAPH. CELLS
STEP - 2
SENSITIZED STAPH. CELLS
STREP. BROTH CULTURE
CO - AGGLUTINATION

FIG. III-B

## APPENDIX III - REFERENCES


1. Koshi, G., Brahmadathan, K.N., Thangavelu, C.P., and Rajeswari Pandian (1979). Evaluation of different methods for the transport of streptococci. Indian J Med Res. 69: 26-31

2. Koshi, G., Rajeswari, K., and Philipose, L. (1977) Preservation of streptococci and other bacteria by sand desiccation and filter-paper techniques. Indian J Med Res. 65: 500-502

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Personal communication.

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Laboratory procedures adopted by Streptococcal laboratory, Centers for Disease Control, Atlanta, Georgia. U.S.A.

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6. Koshi, G., Rajeswari Pandian, and Thangavelu, C.P. (1980). Advantages of Co-agglutination over capillary precipitation technique for grouping of streptococci. Indian J Med Res. 72: 332-340

# APPENDIX IV

## SEROLOGICAL TECHNIQUES

### 1. Anti-streptolysin O (ASO) titration

This was carried out by the microtitre technique as reported earlier[1]. However, the reagents, namely streptolysin O (SLO) antigen, ASO standard etc. were prepared in this laboratory for the latter part of the present study.

Preparation of SLO

Strain: Streptococcus pyogenes, group A C203S strain, kindly supplied by Dr. J. Rotta, WHO Collaborating Centre for Streptococcal Research, Prague, Czechoslovakia.

Medium: (Kalbak's broth)

1000 g of fat free beef heart was minced and mixed with 2 litres of distilled water and kept overnight at 4°C. The next day this was boiled for 10 minutes and filtered through gauze. To the filtrate was added 40 g of Neopeptone (Difco), pH was adjusted to 7.3 and the broth was sterilized by filtration through a memberane filter.

Salt solution consisting of 5 g glucose, 5 g $NaHCO_3$, 5 g NaCl and 2.5 g $Na_2HPO_4 . 12H_2O$ in 100 ml of distilled water was separately sterilised by filtration through a membrane filter. 40 ml of the salt solution was added to 1 litre of the heart infusion just before inoculation of the culture.

Procedure for SLO preparation

One lyophilised culture of strain C203S was opened and the contents transferred aseptically into 50 ml of THB and incubated at 37°C. After 8 hours, when growth was visible by turbidity, this was checked for purity by examining a gram stained smear. If pure, the culture was transferred to 1 litre of Kalbak's broth and incubated at 35°C for 15 hours.

The batch culture was then checked for hemolysin by removing about 2 ml broth culture and centrifuging to sediment the cells. The supernatant was then serially diluted, in 0.5 ml amounts in test tubes. To each broth dilution was added an equal amount of 5% RBC suspension, mixed well and incubated at 37°C for 45 minutes. The tubes were then centrifuged and haemolysis was observed.

When hemolysin was present in dilutions of 1:16 or more, the batch culture was centrifuged and the clear supernatant was filtered through a membrane filter. To the filtrate was added thiomersal to a final concentration of 1:10,000 and this was stored at 4°C for one month. During this period any streptolysin S that have been present would have become inactive.

SLO Standardisation

| | | | |
|---|---|---|---|
| Buffer: | NaCl | - | 9.0 g |
| | $KH_2PO_4$ | - | 3.17 g |
| | $Na_2HPO_4$ | - | 1.81 g |
| | Distilled water | - | 1000 ml |

Dissolve the ingredients in distilled water and adjust pH to 6.3–6.5 This solution can be stored at 2–8 °C. If it reveals turbidity or mold growth, it is discarded and a fresh solution prepared.

Alsever's solution

| | | |
|---|---|---|
| Sodium chloride | – | 4.2 g |
| Sodium citrate | – | 8.0 g |
| Citric acid | – | 0.55 g |
| Glucose | – | 20.5 g |

Add enough distilled water to these salts to make up the volume to 1000 ml. Sterilize by steaming without pressure, three times each for 30 minutes.

Erythrocyte suspension

Mix freshly drawn rabbit blood (cardiac puncture or from ear vein) with Alsever's solution. Wash thrice in buffer or till supernatant is not blood tinged. Centrifuge at 600 g for 10 minutes each time. Suspend cells to make 5.0% suspension. Blood may usually be preserved upto a week in Alsever's solution.

ASO standard

Either the ASO standard produced by the WHO Collaborating Centre for Reference and Research on Streptococci, Prague or the CMCH standard, checked and sanctioned by the WHO Collaborating Centre, Prague was employed.

The lyopilised ASO standard was dissolved in appropriate quantitites of buffer with BSA to yield a final concentration of ASO at 1 unit/ml. The reducing agent sodium dithionite ($Na_2S_2O_4$) was added to the ASO standard at a final concentration of 0.07% (i.e. 7 mg per 10 ml) just before use.

Procedure

Protocol

Ist row (auxiliary tubes) - SLO dilution

| Tube No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| SLO ml | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Buffer ml | 0.5 | 0.75 | 1.0 | 1.25 | 1.5 | 1.75 | 2.0 | 2.25 | 2.5 | 2.75 |
| Dillution | 1.2 | 2.5 | 3.0 | 3.5 | 4.0 | 4.5 | 5.0 | 5.5 | 6.0 | 6.5 |

2nd row (test row)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SLO from auxil. dilution ml | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Std ASO 1 Unit/ml | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |

Mix and incubate in 37 °C waterbath for 15 minutes.

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Add RBC 5% ml | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |

Mix and incubate in 37 °C waterbath for 45 minutes.

Shake after the first 15 minutes of incubation with RBC.

Centrifuge at 1500 rpm for 1 minute.

Record the reading and assess the end-point.

First step: Dilution of SLO

a) Add 0.5 ml of SLO into each of 10 test tubes in 1st row

b) Add buffer as in scheme below

Second step: Test proper

c) From each tube of the 1st row of auxiliary dilutions, transfer 0.5 ml into the corresponding tube in the second row.

d) To the diluted SLO in the second row, add 1 ml of standard ASO diluted with buffer in such a way that 1.0 ml contains 1 unit of ASO and 0.07% of $Na_2S_2O_4$.

e) Incubate at 37°C for 15 minutes.

f) Add 0.5 ml of 5% suspension of rabbit RBC into each tube and mix.

g) Incubate at 37°C for 1 hour.

h) Keep at room temperature for 20 min. or centrifuge for 1 min.

i) Record the result and assess the end-point.

The earlier tubes which contain higher units of streptolysin O will show complete lysis of RBC.

The first tube with complete inhibition of the lysis of RBC (sedimented RBCs and clear supernatant) showing binding of SLO with ASO standard, gives the end-point. The dilution of SLO in this tube gives the working dilution needed for ASO determination by visual technique. Dilute SLO accordingly and use in 0.5 ml quantities in ASO test.

ASO titration

The microtiter technique of ASO titration was carried out. For details refer Koshi (1971)[1].

2. Anti-deoxyribonuclease B (ADNB) titration

Group A streptococci produce four antigenically different deoxyribonucleases (as extracellular enzymes) types A, B, C and D. The most important is the type B enzyme which is produced in large amounts by almost all strains as compared to the production of other deoxyribonuclease types. It is a potent antigen and specific antibodies develop in a high percentage of humans suffering from streptococcal infections and/or their sequelae. A significant rise of this serum antibody is important evidence of an antecedent streptococcal infection. This is of special significance in diagnosis and follow-up of streptococcal skin infections where in most instances ASO fails to rise. The assessment of ADNB is of particular importance in cases of acute glomerulonephritis seen in sub-tropical and tropical areas frequently preceded by infection of the skin. It was also found that elevated anti-deoxyribonuclease B titre persists sometimes for a longer period than anti-streptolysin O level (e.g. in chorea).

Principle

The test is based on the formation of a coloured complex between DNA and methyl green under the given conditions of the reaction. Digestion of the substrate by DNase B is accompanied by loss of colour. If antibodies are present in the serum they prevent the enzyme from attacking the substrate and the coloured complex is retained. The ADNB titration is done by a micro-method, similar to that described by Nelson et al.[2].

## Materials

### Buffers

1) 0.1 Tris-HCl buffer, pH 7.8 with 0.02 M $Mg^{2+}$ ions Dissolve 1.21 g of tris-hydroxymethylaminomethane in 90 ml of distilled water, adjust pH to 7.8 with 1 N HCl, add 0.49 g of $MgSO_4 \cdot 7H_2O$ and complete to volume 100 ml;

2) 0.01 M Tris-HCl buffer, pH 7.8, with 0.001 M $Ca^{2+}$ ions, 0.001 M $Mg^{2}$ ions and 0.01% of bovine-serum albumin. Dissolve 1.21 g of Tris-hydroxymethylaminomethane in 900 ml of distilled water, adjust pH to 7.8 with 0.1 N HCl, add 0.219 g of $CaCl_2 \cdot 6H_2O$, 0.246 g of $MgSO_4 \cdot 7H_2O$ and 1 g of bovine serum albumin and complete with distilled water to a volume of 1000 ml.

3) 0.02 M acetate buffer, pH 4.2 Dissolve 136 mg of sodium acetate in 40 ml of distilled water, adjust pH to 4.2 with 1 N HCl and complete the volume to 50 ml.

Microtiter system (Cook Eng. Co., Alexandria, Virginia, USA)

The apparatus consists of:-

1. Microtiter "U" plates
2. 0.025-ml pipette dropper
3. 0.050-ml pipette dropper
4. 0.025-ml microdiluter
5. test-reading mirror (not absolutely necessary)

### Substrate

A 0.1% solution of deoxyribonucleic acid (DNA) with 0.01% of methyl green.

1. Dissolve 100 mg of methyl green (Fisher Certified Reagent, C.I.No.42590, Fisher Scientific Co. USA) in 10 ml of buffer 3. Extract repeatedly with chloroform (in separatory funnel, using two volumes of chloroform) until the chloroform layer no longer displays a red-violet colour (removal of crystal violet which may be a side-product in the stain solution). After removal of the chloroform layer, the solution is storable at +4 °C for several months.

2. To 100 mg DNA (from calf thymus, highly polymerized, sodiumsalt, type V, Sigma Co., St. Louis, Mo., USA), add 50 ml of distilled water and a few drops of chloroform as preservative. Allow to swell for 30 minutes at +4 °c, then stir approx. 15 minutes at room temperature. Keep at +4 °C overnight. To be sure that all DNA has come into solution stir for another 15 minutes at room temperature.

3. While stirring the DNA solution, add 50 ml of buffer 1 followed by 1 ml of methyl-green solution. Mix thoroughly and keep at approx. 20 °C (in air-conditioned room or outside but close to the cold room etc.) for 24 hours (to allow the DNA - methyl-green complex to form). The solution may then be stored at +4 °C for about 1 week.

It is also possible to use a freeze-dried DNA - methyl-green complex. The solution is prepared by dissolving the lyophilizate in original volume of distilled water (keep at room temperature approx. 20 °C for about 24 hours).

ADNB standard

Use serum of known ADNB titre. An international standard is being prepared and will be available in the near future.

Antigen

Streptococcal deoxyribonuclease B (DNase B) prepared from culture supernate of reference strain C203S, as follows:-

(1) Use Todd-Hewitt broth throughout. Always use a new vial of freeze-dried culture. Select a few colonies from a blood agar plate to inoculate 10 ml of prewarmed broth and incubate for 8 hours. Add this preculture to 1000 ml of broth and incubate at the same temperature for approx. 18 hours. After checking for purity, filter through bacterial filter at +4 °C.

(2) Add ammonium sulphate under stirring to culture filtrate to 57% saturation (430 g per 1000 ml) at 20 °C. Allow precipitate to form at +4 °C for approx. 18 hours and then spin it off at 2500 rpm for 20-30 min. Dissolve the precipitate in distilled water (approx. 5% of the original volume) and dialyse against distilled water at +4 °C, stirring and occasionally changing the dialysis fluid until ammonium ions are removed (test e.g. with Nessler's reagent; brown precipitate signifies positive

reaction). On completing the dialysis, a sample of the crude enzyme is withdrawn for activity assay and the rest distributed in 10 ml volumes and stored at $-60^{o}$ C.

(3) Dilute the enzyme 1:100. Using a 0.025 ml pipette dropper and 0.025 ml of buffer 2 into each of wells 1-10. Using the 0.025 ml micro-diluter add 0.025 ml of diluted enzyme into well 1, mix and transfer equal volumes of mixture consecutively from well to well. A series of enzyme dilutions (geometrical progression, quotient=2) ranging 1:200 to 1:10240 is thus prepared. Then add, using pipette droppers, 0.025 ml of buffer 2 and 0.050 ml of substrate into each well. Mix by tapping the edge of the plate and incubate at $37^{o}$ C in the incubator for 18-20 hours. (To prevent evaporation, place the microtiter plates in a wet chamber during the incubation or overlay them with parafilm, etc). The reciprocal value of that enzmyme dilution which has produced approx. 50% digestion of the substrate (coloring at 2+, see later) is read as the end point. Only that preparation of the enzyme showing a titre of over 1600 is suitable for further purification.

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4) Purification of enzyme by diethyl-aminoethyl (DEAE) Cellulose chromatography

a) Chromatographic column: 2.5x28 cm packed with DE cellulose 52 Whatman, U.K.

b) Buffers: 0.004 glycine-NaOH buffer, pH 9.0, with 0.008 M NaCl Dissolve 0.3 g of glycine in 900 ml of distilled water, adjust pH to 9.0 with 0.1 N NaOH, add 0.47 g of NaCl and complete to volume 1000 ml; 0.004 M glycine-NaOH buffer, pH 9.0, with 1 M NaCl (Dissolve 58.5 g of NaCl in 1000 ml of buffer).

c) Preparation of column: Add approximately 15 volumes of 0.1 N NaOH to preswollen cellulose, stir and let stand for approx. 30 min. Discard the supernate (preferably by means of an air pump) and wash with distilled water until neutral reaction. Discard the supernate, overlay the cellulose with 15 volumes of 0.1 N HCl, stir and let stand for 30 min. Discard supernate and again wash with 0.1 N HCl under identical conditions and with distilled water until neutral reaction. Discard the distilled water, suspend the cellulose in glycine buffer containing 0.008 M NaCl and cool at +4 °C. Then pack the chromatographic column, kept at +4 °C, with the cellulose cycled as described and stabilized by washing with identical buffer (at least 50 ml) at +4 °C.

d) Chromatography: Apply 10 ml of crude enzyme (see point 3) to the cellulose column and elute with 0.004 M glycine buffer containing 0.008 M NaCl. Add 0.1 ml of a 0.5% water solution of bovine-serum albumin to each of the fraction collecting test tubes as stablizer for the eluted protein. Regulate the flow rate so that separate fractions amounting to approx. 5 ml each would collect at 7-10 min. intervals. If facilities for automatic "on-the-run" recording of the elution curve are not available, analyse the individual fractions by measuring in UV light, at 280-nm. The first peak, which is eluted soon after the void volume, cointains DNase B.

After completing the chromatographic separation of DNase B elute the proteins retained by the column with 1 M NaCl glycine buffer.

Collect samples from each of the first-peak fractions for enzyme activity assays and store the remainder at $-60^{o}$ C.

After precisely determining the activities (see below) of the individual fractions, pool the peak fractions displaying the highest activity, if necessary diluting with buffer 2 to obtain a resulting enzyme titre about 400 units. Distribute the enzyme in precise 0.3-0.5 ml aliquotes and freeze-dry or store at $-60^{o}$ C.

e) Titration of Chromatographically purified DNase B:

Dilute the ADNB standard with buffer (2) so that it contains 1 unit in 1 ml (e.g. ADNB with a titre of 480 units is diluted 1:480). Dissolve a freeze-dried sample of the enzyme in an amount of distilled water corresponding to its original volume. Dilute the enzyme 1:50, 1:100, 1:200, 1:300.....1:1000 in test tubes. Using a 0.025 ml pipette dropper, transfer 0.025 ml of diluted enzyme from each test tube into the respective wells (begin with the highest enzyme dilution). Add 0.025 ml of diluted standard ADNB into each well and after mixing (by tapping at the plate edge) incubate at 37 °C in the incubator for 15 min.; then add 0.050 ml of substrate to all wells with a pipette dropper, mix and incubate under identical conditions for 18-20 hours; mix again after first 15 min. of incubation.

Titre of the enzyme is the reciprocal value of the highest enzyme dilution that in the presence of antibody causes approx. 50% digestion of the substrate (colouring 2+). To determine the enzyme titre exactly, it is necessary to repeat the above-described titration against the standard a number of times, using narrower ranges of enzyme dilutions.

The colour intensity for determining the titre of the enzyme is read as follows:-

3+, no digestion of substrate;

2+, approximately 50% digestion;

1+, over 50% digestion;

-, colourless, complete digestion of substrate

Anti-DNase B( ADNB) titration

Inactivate patient's serum at 56 °C for 30 min.

Dilute the serum specimen to be tested in test tube 1:25. From this dilution, prepare dilutions of 1:50 and 1:75. The sera are titrated in the horizontal, rows of the microtiter plate. Add 0.025 ml of buffer( 2) into all the wells of the plate using a pipette dropper. Use a 0.025 ml microdiluter to transfer an equal volume of serum diluted 1:50 into the first well ( be careful to dip only the diluter head) and after mixing by rotatory motion, transfer the same volume into the next well. Proceed thus in the horizontal row from well 1 to 6. Similarly, prepare serum dilutions from the starting dilution 1:75 in wells 7-12. In this way you will obtain two rows of serial serum dilutions with a quotient of 2 ( serum dilutions of 1: 100 to 1:3200 and 1: 150 to 1:4800). Using a 0.025 ml pipette dropper, to each well add apropriately diluted enzyme solution ( e.g. enzyme titre is 325 units in 1 ml: dilute 1:325 for antibody titration) prepared immediately before use. Mix throughly and incubate at 37 °C for 15 min. The rest of the procedure is similar to that described for titration of purified enzyme.

Controls

a) Enzyme control: 0.025 ml of buffer(2) 0.025 ml of diluted enzyme and 0.05 ml of substrate solution (must be colourless after incubation).

b) Substrate control: 0.05 ml of buffer(2) and 0.05 ml of substrate solution (must green colour after incubation).

Titre determination

The ADNB titre corresponds to the reciprocal value of that serum dilution which causes an approx. 50% inhibition of the enzyme (colouring 2+); if there is a steep colour transition (colour 3+ and next total loss of colour), read the last well with total enzyme inhibition (colour 3+) as the end point.

At Vellore, the Geometric mean titre (GMT) of ADNB has been estimated in different subject groups and found as 400 units. Two dilutions above the GMT, namely, 800 units and over is considered as elevated ADNB titre.

3. Anti-hyaluronidase (AH) titration

This test was carried out with commercially available reagents (Difco, U.S.A.). The test, based on mucin-clot prevention technique, was carried out by the mmicrotitre method, as described by Murphy[3].

The microtitre modification of the AH test involved the incorporation of India ink (carbon particles) along with the substrate, i.e. Potassium hyaluronate. For this, 0.01 ml of India ink was added to 20 ml of distilled water and mixed well; this was used for rehydration of the substrate.

The dilution scheme, incubation time and other steps involved in the micromethod were the same as recommended by the manufacturer for the tube method, except that the volume of reagents was reduced (e.g. 0.025 ml of serum dilutions, instead of 0.25 ml)

Results and interpretation

The reciprocal of the highest dilution of serum showing formation of a firm black clot was recorded as the AH titre of the specimen.

Titres of 1024 and above were considered as elevated, and hence indicative of a recent streptococcal infection.

4. C-reactive protein detection

C-RP was detected by a capillary precipitation technique originally described by Anderson and McCarty[4].

Reagents for this test, namely C-RP antiserum, C-RP control serum, etc., were purchased from Difco, U.S.A.

Technique

Instructions of the manufacturer were followed:-

1) A glass capillary 90 mm in length by 0.7-1.0 mm internal diameter was used to draw the C-RP antiserum to about a third of its length.

2) The outside of the capillary was cleaned with a tissue paper and then it was dipped into the test serum sample and an equal volume allowed to enter in. Care was taken to avoid air bubbles at the interface.

3) The capillary tube was inverted several times to ensure mixing of the reagents.

4) The fluid column was moved to the centre and the lower end of the tube was inserted into plasticine in the groove of a wooden block and the tubes kept erect after sealing at the upper end with plasticine.

5) A positive control was always included with the test samples.

6) All capillaries were then incubated at 37 $^{o}$C for 2 hours and then held at 4 $^{o}$C overnight.

Reading

The height of the precipitate formed was measured in millimeters and the results expressed as 1+ to 6+ corresponding to 1 mm through 6 mm.

## REFERENCES - APPENDIX IV


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## APPENDIX V

## PRODUCTION OF STREPTOCOCCAL ANTISERA

Technique, as followed by streptococcal laboratory, Centres for Disease Control (CDC) Atlanta, Georgia, U.S.A. were followed.

### 1. GROUPING ANTISERA

Antisera against streptococcal groups A,B,C,D,F and G were raised in locally procured adult rabbits by immunisation with vaccines prepared from standard streptococcal strains supplied by Dr. Max M. Moody, formerly, Chief of Streptococcallaboratory and few from Dr. R. Facklam present Chief of streptococcal laboratory, C.D.C., Atlanta, Georgia.

#### Preparation of vaccine for immunisation

The following reference strains were employed for vaccine production.

| Group | Strain No.(C.D.C) |
|---|---|
| A | SS103 |
| B | SS617 |
| C | SS188 |
| D | SS498 |
| F | SS727 |
| G | SS13 |

#### Pepsin treated Vaccine for group A, C and G

1) Inoculate 5 ml amounts of THB supplemented with a few drops of sterile defibrinated sheep blood with a pure culture of the reference strain and incubate at 37 $^{o}$C in a

waterbath 5 to 6 hours.

2) Transfer two to three drops of this blood broth culture into each of six 200 ml centrifuge bottles containing 175 ml of THB each. These are incubated overnight at 37°C.

3) Subculture loopfuls from each bottle on blood agar plates to check purity.

4) Centrifuge each bottle and discard the supernatant. Refrigerate the cells overnight.

5) Check purity plates, if pure, combine cells from all bottles in 25ml of sterile physiological saline.

6) Add 0.25 ml 1N HCl and 0.25 ml of 10% Pepsin solution, prepared in N/100 HCl and sterilized by filtration (equals 1 mg pepsin per ml of cell suspension). Adjust pH to 2.0 and incubate at 37°C for 2 hours.

7) Sediment cells by centrifugation and discard supernatant.

8) Resuspend bacterial sediment in 50 ml saline, neutralise and then centrifuge to sediment. Wash once more with sterile saline.

9) Resuspend washed cells in 15 ml of 0.4% formalinized saline.

10) Remove 2 ml of this suspension, and extract the group specific antigen by acid heat method. Use 1N HCl and NaOH instead of N/5 solutions to adjust the pH, and do not centrifuge the suspension before extraction. Test by capillary precipitation with reference antisera.

11) If the test extract reveals precipitation with hemologous reference serum, store the stock vaccine at 4°C. Dilute 1:10 with saline for vaccination of animals.

Formalin killed Vaccine for group B

1) Inoculate 5 ml TH blood broth with stock culture of the immunising strain. Incubate in a waterbath at 37°C for 5 to 6 hours.

2) Transfer 2 to 3 drops from this actively growing culture to three 250 ml centrifuge bottles containing 175 ml THB. Incubate at 37°C overnight.

3) Subculture loopfuls on blood agar plate to check for purity.

4) Prepare hot-acid extract from cells of one bottle and check by precipitation test, with reference antiserum.

5) Resuspend cells from 2 bottles in 12 ml of freshly prepared 4% formalinized saline and refrigerate overnight.

6) Subculture 0.1 ml of formalinized cell suspension on blood agar plate to check for sterility. Incubate the sterility plate at 37°C for 48 hours.

7) Store stock vaccine at 4°C.

8) Dilute the stock vaccine 1:20 with saline before immunisation. Make fresh dilutions of the vaccine each day.

Glucose enriched, formalin killed Vaccine for Group D

1) Inoculate 5 ml of TH blood broth with a pure culture of the immunising strain. Incubate at 37°C in a waterbath for 5-6 hours.

2) Transfer 2-3 drops of the actively growing culture to each of three 250 ml centrifuge bottles containing 175 ml THB each. To each bottle, aseptically add 3.5 ml of a 50% glucose solution which has previously been sterilized by filtration.
Incubate at 37°C overnight.

3) Subculture loopfuls on blood agar plate to check purity.

4) Sediment cells by centrifugation and discard supernatant.

5) Prepare hot-acid extract from cells of one bottle and test by capillary precipitation test with reference antiserum.

6) If the extract gives satisfactory result, resuspend cells from two bottles in 12 ml of freshly prepared 4% formalinised saline. Refrigerate overnight or until sterility plates show no growth. (It may take 4 to 5 days).

7) Subculture 0.1 ml of formalinised cell suspension on blood agar plate to check sterility. Continue incubation of plate at 37°C for 48 hours.

8) Store stock vaccine at 4°C.

9) Dilute stock vaccine 1:20 with saline, before vaccination of animals. Prepare fresh dilutions each day.

Heat killed Vaccine for group F

1. Inoculate 5 ml THB with a pure culture of the immunizing strain. Add two drops of supplement (7.5 g sodium bicarbonate and 15.0 g glucose dissolved in 100 ml of distilled water and sterilized by filtration under positive pressure). Incubate at 37°C in a waterbath for 5 to 6 hours. The supplement eliminates the need for incubating group F strains under $CO_2$.
2. Transfer 2 to 3 drops of the actively growing culture to three 250 ml centrifuge bottles containing 175 ml THB each. Add 12 ml of supplement to each bottle. Incubate at 37°C overnight.
3. Subculture loopfuls on blood agar plate to check for purity.
4. Sediment cells by centrifugation and discard supernatant fluid.
5. Prepare hot-acid extract from cells of one bottle and check by capillary precipitation with reference antisera.
6. If the extract reveals satisfactory results, resuspend cells from two bottles in 12 ml saline. Mix and heat in a 56°C waterbath for 30 min. DO NOT KILL WITH FORMALIN.

7. Streak loopfuls on blood agar plate to check sterility. Incubate sterility plates at 37°C for 48 hours.

8. Store stock vaccine at 4°C.

9. Dilute stock vaccine 1:20 with sterile physiological saline, for vaccination of animals. Prepare fresh dilutions each day.

Vaccination schedule

For groups A, C and G

First week: One intravenous (IV) inoculation 0.5 ml each on 3 consecutive days (Monday, Tuesday and Wednesday).

Second, third and fourth weeks: One IV inoculation of 1.0 ml on weeks each of three successive days.

Monday-Test bleed. If the serum gives strong precipitation within 5 min. with extracts of several strains belonging to the homologous group, bleed the animal to death. (For group C, at least one human and one animal strain should be used to check the antiserum). If the results of test bleeding is unsatisfactory, continue immunization for two more weeks and then recheck.

For groups B, D and F

First week: Three IV inoculations of 0.5 ml each, 2 IV inoculations of 1.0 ml each on successive days.

Second week: Five IV inoculations of 1.0 ml on successive days (Monday through Friday)

Third week: Two IV inoculations of 1.0 ml each on Monday and Tuesday.

Fourth week: Test bleed and proceed as described for groups A, C and G.

Second or subsequent series of vaccination

If the rabbits have not responded after 18 inoculations, rest them for 2 or 3 months and give booster inoculations with freshly prepared vaccine.

First week: Three subcutaneous inoculations of 0.5 ml each on successive days (for Group A,C and G)

For groups B, D and F

Three subcutaneous and two IV inoculations of 0.5 ml each on successive days.

Second week: For A, C and G

Three IV inoculations of 0.5 ml each on successive days.

For B, D and F

Test bleed and check.

Third to fifth weeks For A,C, and G only

Continue as for first series. Test bleed and check.

EVALUATION AND CHECKING:

1) Test each antiserum by capillary precipitation with hot-acid extract of multiple strains of different serological groups, especially of those groups which

are known to have type-specific antigens (groups B, D and F).

2) Antisera that react with extracts of all strains of the homologous group alone should be considered satisfactory.

3) Select antisera that show no cross reactions with extracts of heterologous group strains.

4) If necessary, antisera that show cross-reactions with other groups may be used after absorption of the cross-reacting antibodies.

ABSORPTION OF ANTISERA

Absorbing cells:

1) Determine the strain to be used for absorption of each batch of antiserum by absorbing small amounts (1.0 ml) with strains that reveal cross-reactions (one strain at a time) until a strain is found which removes all cross-reacting antibodies.

2) Grow the cells to be used for absorption in a large volume of THB (usually 1 to 4 litres).

3. To increase cell yield, add supplement composed of 7.5% sodium bicarbonate and 15% sucrose (prepared in distilled water and sterilised by filtration under positive pressure) at the rate of one volume per 20 volume of culture.

4. Check purity and heat-kill the cells at 56 $^{o}$C for 1 hour in a waterbath.

5. Check sterility.

6. Centrifuge and resuspend the sedimented cells in thrice their volume of sterile physiological saline.

7. Add merthiolate to a final concentration of 1:10,000 and store at 4°C until needed.

Absorption procedure

1. Determine the ratio of cells to serum to be used for absorption, by absorbing small amounts (1.0 ml) of serum with different volumes of absorbing cells. Find the least amount of cells which will remove the cross-reactions. The ratio may vary from 1 part packed cells to 1 part antiserum to 1 part packed cells to >10 parts of antiserum

2. Pack the appropriate volume of absorbing cells required by centrifugation. Discard supernatant.

3. Thoroughly mix cells and antiserum with a pipette and incubate at 37°C for 50 minutes.

4. Separate serum from cells by centrifugation.

5. Decant antiserum and test by capillary precipitation. If cross-reacting antibodies are still present, repeat the absorption procedure.

CLARIFICATION AND STERILIZATION OF SERUM

Sera which were chylous or that developed cloudiness after being at 4°C for sometime, were clarified by treatment with chloroform. Only those antisera which were ready for use were clarified. The clarification was carried out as follows:-

6. Centrifuge and resuspend the sedimented cells in thrice their volume of sterile physiological saline.

7. Add merthiolate to a final concentration of 1:10,000 and store at 4°C until needed.

Absorption procedure

1. Determine the ratio of cells to serum to be used for absorption, by absorbing small amounts (1.0 ml) of serum with different volumes of absorbing cells. Find the least amount of cells which will remove the cross-reactions. The ratio may vary from 1 part packed cells to 1 part antiserum to 1 part packed cells to >10 parts of antiserum

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1) Two volumes chloroform were added to one volume of antiserum in a beaker.

2) Both were mixed with an `ultra-turrex' mixer at low speed for three intervals of 5 minutes each. The antiserum was allowed to separate from the chloroform between the intervals (about 1/2 to 1 min).

3) The mixture was refrigerated overnight.

4) Chloroform layer was separated from under the antiserum with the use of a Pasteur pipette.

5) The antiserum was centrifuged at 10,000 g for 1 hour.

6) Clear antiserum was decanted into a sterile tube and held at 4 $^{o}$C for one week.

7) Antiserum was recentrifuged again at 10,000 g for 1 hour.

8) The clarified serum was sterilised by filtration through a membrane filter or through a porcelain filter candle.

Preservation and storage

Merthiolate was added to a final concentration of 1:10,000 as a preservative.

Unabsorbed, unclarified sera were stored at 4 $^{o}$C for a few months.

Absorbed and clarified sera were either lyophilised (courtesy of the IVPM, Ranipet) or stored at -60 $^{o}$C.

## II. ANTISERA FOR T-TYPES OF GROUPS A STREPTOCOCCI

### Strain for Immunisation

Standard cultures containing known T antigens were used. These strains were kindly supplied by Dr. Max M. Moody, Centers for Disease Control, Atlanta, U.S.A. The T-type and the code number of these strains are as follows:-

| | Strain Code |
|---|---|
| T-type 1 | - SS.566 |
| T-type 2 | - SS.567 |
| T-type 3 | - SS.568 |
| T-type 4 | - SS.569 |
| T-type 5 | - SS.570 |
| T-type 6 | - 8/49 WHO (Courtesy, Dr. Rotta, Prague) |
| T-type 8 | - SS.572 |
| T-type 9 | - SS.573 |
| T-type 11 | - SS.574 |
| T-type 12 | - SS.575 |
| T-type 13 | - SS.576 |
| T-type 14 | - SS.577 |
| T-type 22 | - SS.579 |
| T-type 23 | - SS.580 |
| T-type 25 | - SS.581 |
| T-type 27 | - SS.582 |
| T-type 28 | - SS.583 |
| T-type 44 | - SS.584 |
| T-type B 3264 | - SS.585 |
| T-type Imp 19 | - SS.586 |

VACCINE PREPARATION:

1. Inoculate a pure culture of the immunising strain into 5 ml THB and incubate at 37°C for 4 to 6 hours.
2. Transfer 4 to 5 drops of this rapidly growing culture to 175 ml of THB containing 1% trypsin (Difco., 1:250). Incubate overnight at 30°C, or if needed at 22°C.
3. Subculture on blood agar plate for purity.
4. Sediment cells by centrifugation and resuspend in 0.01 M, phosphate buffer, pH 7.8, containing 5% trypsin.
5. Incubate overnight at room temperature.
6. Wash cells six times with 0.85% saline.
7. Remove a small aliquot of cell suspension (ca 3 ml) after the last wash and prepare a T suspension, from 1 ml of suspension. Check for T antigen by slide agglutination with homologous reference type sera. The antigen should give at least a 2+ agglutination. Prepare an acid heat extract from the remaining 2 ml of the cell suspension and check the absence of M antigen, by capillary precipitation test.
8. If the cells give a strong agglutination with homologous type serum, resuspend the washed cells in 3% formalized saline. (The cells recovered from a 175 ml THB culture should be resuspended in 25 ml of formalinised saline). Keep at 4-10°C for 2 to 3 hours.
9. Subculture on blood agar plates to check sterility.

10. After the vaccine has been killed, sediment the cells by centrifugation, and replace half of the formalinized saline to reduce the formalin content to 1.5% for immunisation.

IMMUNIZATION SCHEDULE:

First week:

Inoculate 0.5 ml intravenously. (Preferably on the second day (Tuesday) of the week).

Second through fourth week:

Inoculate 1.0 ml IV on each of three successive days per week (i.e. on Monday, Tuesday and Wednesday).

Bleeding:

Test bleed 6 to 8 days after the last inoculation

Dilute the serum 1:5 with 0.85% saline and absorb with T-type 6 cells. (Use type 44 cells for type 6 antiserum) by mixing 1 vol.of packed cells with 3 volumes of diluted serum and incubating 37 $^{o}$C temperature for 30 minutes to 1 hour.

Centrifuge to sediment the cells and test serial dilutions of the antisera by slide agglutination with homologous T antigen. If the serum gives a 2+ agglutination at 1:160 dilution or higher, the rabbit may be bled by cardiac puncture.

If the titre is low, continue vaccination with 1.0 ml of antigen given IV on each of 3 successive days per week.

Recheck after 8 weeks of immunisation. If still low, discard the rabbit.

## ABSORPTION OF T-TYPE ANTISERA

All T antisera should be absorbed with type 6 cells (type 6 anti-sera should be absorbed with type 44 cells). Some antisera might need additional absorptions with other heterologous cells.

### Absorbing cells:

1. T-type 6 cells are grown in bulk volumes of THB, as described under grouping antisera production. The culture is usually incubated for 48 hours.
2. Subculture on blood agar to check for purity and then heat kill the cells at 60 °C for 1 hour in a water bath.
3. Wash cells three times in sterile saline and resuspend 0.02 M phosphate buffer pH 7.8.
4. Boil cell suspension for one hour and then store at 4 °C.

### Absorption procedure:

1. Dilute antisera 1:5 with saline.
2. Mix one volume of packed absorbing cells with three volumes of diluted antisera and incubate at 37 °C for 1 hour.
3. Centrifuge and remove the serum from the cells.
4. Test the antisera by slide agglutination with absorbing cells. If a positive reaction occurs, repeat the absorption.

Evaluation:

Check each antisera by slide agglutination with all T antigens. The absorbed antisera should give at least a 2+ agglutination with homologous T antigen, when diluted in 1:160. Except for known complexes, the absorbed serum should not react with heterologous antigen.

Preparation of antiserum pools

Combine equal volumes of satisfactory antisera as follows:

| Pool | Individual types | | | |
|---|---|---|---|---|
| T | 1 | 3 | 13 | B 3264 |
| U | 2 | 4 | 6 | 28 |
| W | 5 | 11 | 12 | 27 44 |
| X | 8 | 14 | 25 | Imp 19 |
| Y | 9 | 22 | 23 | |

Preservation and Storage

Preserve unabsorbed sera by addition of merthiolate of final concentration of 1 in 10,000 or sodium azide at 0.02% concentration.

Absorb only small aliquot of sera as needed. The absorbed sera can be stored at 4 $^{o}$C for a few months or may be lyophilized.

# APPENDIX VI

## PRESERVATION OF STREPTOCOCCAL STRAINS, SERA AND REAGENTS

### Streptococcal strains

### Storage in Robertson's Cooked Meat (RCM) Medium

Inoculate multiple colonies from a pure culture on blood agar plate into RCM and incubate for 24 hours. Seal the tube with sterile cork dipped in sterile melted wax. These can be stored at 4-8 $^{o}$C for 36 months.

### Storage on Filter Paper strips

Pure cultures of streptococcal isolates may be transferred on to filter paper strips, dried in air and preserved for a period of about 3 weeks. Under high temperature and humidity, groups A and C may survive only for 10 days and B and G for 14 days[1].

### Storage in Blood Broth

Inoculate multiple colonies from a pure culture on BA into 5.0 ml Todd-Hewitt (TH) broth enriched with 0.25 ml (5%) defibrinated sheep blood. Incubate for 24-48 hours. It is ideal to have these broths in screw-capped test tubes. Alternately, the 4x1/2" test tubes may be sealed with sterile cork, dipped in wax. Store the cultures at 4-8 $^{o}$C and subculture into new media once in two weeks.

## Storage by sand desiccation

Storage by sand desiccation is a simple and easy technique which can be used by even small laboratories with minimum facilities. Streptococci can be preserved for as long as 15 years by this method. We have preserved our stock strains by this technique for 9 years and six months [2,3].

## Procedures

1) Prepare blood agar (BA) slopes, preferably in screwcapped tubes. Just before use, add one drop of sterile defibrinated sheep blood to each tube with a Pasteur pipette.

2) Inoculate a pure culture of the streptococcal isolate onto the BA slope and incubate overnight at 35$^{o}$-37$^{o}$C. For group F streptococci and other fastidious organisms, incubate in a candle jar at 35$^{o}$-37$^{o}$C.

3) Observe the slope for growth or contamination. If pure add 2-3 drops of sterile defibrinated sheep blood over the growth on each slope with a Pasteur pipette.

4) Gently rub the entire surface of the slope with the Pasteur pipette while expelling the blood. Draw the blood and bacteria mixture into the pipette and repeat this process till most of the organisms are suspended in the blood.

5) Draw the blood-bacteria mixture into the Pasteur pipette. Expel this into a sterile Durham tube, containing sterile sand. (These sand containing

tubes are sterilized in an oven at 160 $^{o}$C for 1 hour). Thoroughly coat all the sand particles with blood-bacteria mixture.

6) With the tip of the Pasteur pipette at the bottom of the sand tube, withdraw as much excess blood as possible. Discard the excess into disinfectant solution. It is important to remove as much blood as possible from the sand tube.

7) Before withdrawing the pipette for the last time, gently push the sand particles to the sides of the Durham tube leaving a small core through the middle. This provides a thinner layer for more rapid drying and prevents formation of a hard, stone-like mass of sand which is difficult to manage while subculturing.

8) Push the cotton plug about half way down the tube and clip off any protruding excess cotton, using scissors and then burn the tip off the tube.

9) <u>Desiccation</u>

(a) Place an approximately 1/2 inch thick layer of dessicant (silica gel or calcium sulphate) mixed with indicator gel in the bottom of a Mason jar or vaseline jar and cover this with double layer of cotton gauze. Dry this bottle in an oven at 60 $^{o}$C for about 30 min. to an hour.

(b) Place the blood-bacteria coated sand tubes inside the jar. It is convenient to bundle about 10 tubes together with a rubber band or thread.

(c) Roll some plasticine on the table top to form a cylinder about the thickness of a pencil. Lay this on the lip of the jar and join the ends solidly together. Cover the jar with a flat glass cover, placed firmly on the plasticine.

(d) Using a straightened paper clip, make a hole in the plasticine ring.

(e) Place the jar inside a vacuum desiccator, connected to a good vacuum pump (Fig. VI-A). Evacuate the desiccator jar about 45 min. to 1 hour.

(f) Close the desiccator and stop the pump. Open the valve of the desiccator with a quick action, so that the resulting sudden in-rush of air forces the glass lid of the jar down into the plasticine, thereby effectively sealing the jar.

Storage

Store the jar with sand desiccated culture at 4 $^{o}$C for longer survival of the bacteria.

Recovery of culture preserved by sand desiccation

1) Using a towel or a thick pad of cloth, carefully apply enough force to dislodge the glass cover from the top of the jar. A strong hiss is heard when the vacuum is broken.

2) With a sharp pointed forceps pull the cotton plug partly out of the sand tube. The tube can now be handled as a standard culture tube.

3) Use a straightened paper clip hooked at the tip as an inoculating needle. Sterilize this in a Bunsen flame and plunge into sterile THB to cool and to wet.

4) Place the wet needle into the sand tube and twist it gently until a few grains of sand are loosened and stick to the wet vial.

5) Replace the cotton plug in the sand tube and transfer the sand grains to the tube of THB.

6) Add a few drops of sterile defibrinated sheep blood to the THB culture and incubate at 35$^{o}$-37$^{o}$ C. Most strains grow readily within 16-24 hrs., but a few may require 48-72 hrs. of incubation.

7) Adjust the plug of the sand tube as described under desiccation and return it to the jar. Carefully clear the old plasticine and apply a fresh roll of plasticine on the lip of the jar and the lid. Reseal the jar. If no freshly prepared sand tube is added to the jar, 15 min. of evacuation will suffice.

Storage by lyophilization

Streptococcal strains were lyophilized with the use of an Edwards EF03 freeze drier (refrigerated version). For details of lyophilization technique, please refer to Lapage, S.P. et al. (1970).

In brief, pure cultures of streptococcal isolates were grown overnight in 30 ml THB at 37$^{o}$ C and the cells, sedimented by centrifugation, were resuspended in about 1 ml of 7.5% glucose serum (Lapage, S.P. et al. 1970). A loopful

of this suspension is then subcultured on blood agar (BA) to check for purity. Two to three drops of this bacterial suspension are then dispensed in sterile pre-labeled ampoules and lyophilized.

Recovery of lyophilised cultures of streptococci

Identification of cultures

Identify the cultures by the label on the paper inside the ampoule reading from the round end of the tube (see Fig. VI.B).

Opening of ampoules

(a) Make a file mark on the ampoule about the middle of the cotton wool plug, and apply a red-hot glass rod at that site to crack the glass.

(b) Allow time for air, filtered by the plug, to seep into the ampoule; otherwise when the pointed end is snapped off, the plug will be drawn in. Hasty opening may release aerosols.

(c) The plug should be removed with a forceps. The discarded plug and the pointed end of the ampoule should be put into a lotion jar.

Re-hydration and recovery

(a) Flame the open end of the tube and add about 0.3 to 0.5 ml of Todd-Hewitt broth using a sterile Pasteur pipette.

(b) Mix the contents carefully so as to avoid frothing. Most of the organisms will be on the label paper.

(c) Lift the paper with sterile forceps and place on one half of a blood agar plate(BA).

(d) Subculture a loopful of the broth suspension on the other half of the BA.

(e) Transfer the rest to a tube containing 5 ml Todd-Hewitt broth enriched with a few drops of sterile defibrinated sheep blood.

(f) Incubate both the broth and plate cultures at 35-37 °C. Some cultures may need incubation for several days.

(g) Pick up isolated colonies and proceed to maintain them as stock cultures.

2. Preservation of sera

Streptococcal Grouping sera

These were preserved by addition of merthiolate to a final concentration of 1:10,000 and dispensed in 1 to 5 ml aliquotes in sterile ampoules. These ampoules were sealed and were stored at 4 °C.

Sera that were absorbed to remove cross reactions, were finally filtered through Seitz filter, preserved by addition of merthiolate and were stored as above.

Typing sera

Aliquotes of unabsorbed sera were merthiolated and dispensed in sterile ampoules which were sealed and then stored at 4 °C.

Absorbed sera were sterilized by filtration, preserved by merthiolate and stored at 4 °C. Alternatively, some batches of absorbed sera were lyophilized.

3. Preservation of reagents

Streptolysin O (SLO)

This was preserved by addition of merthiolate to a final concentration of 1:10,000 and was stored at 4°C for as long as two years, without any change in its activity.

Alternatively, aliquotes of 2.5 ml or 5 ml were lyophilized by standard methods. The lyophilized SLO was also stored at 4°C.

Buffer for Anti-streptolysin O (ASO) titration

The phosphate buffered saline, without bovine serum albumin was prepared in bulk (usually 1 or 2 litres) and stored at 4°C for a few weeks. If any signs of contamination, as evidenced by the development of turbidity, or mold growth were observed, the buffer was discarded.

Anti-streptolysin O (ASO) standard

The ASO standard was always preserved by lyophilization.

Precise quantities of the ASO standard were dispensed in sterile ampoules for freeze drying. Final standardisation was carried out after lyophilization. Lyophilized ASO standard was stored at 4°C.

DNase B enzyme

Crude DNase B enzyme, was preserved at -60°C.

Purified DNase B was immediately lyophilized. Great care was taken in dispensing precise, quantities of purified DNase B into the ampoules for lyophilization. Lyophilized, purified enzyme was stored in a desiccater at 4°C.

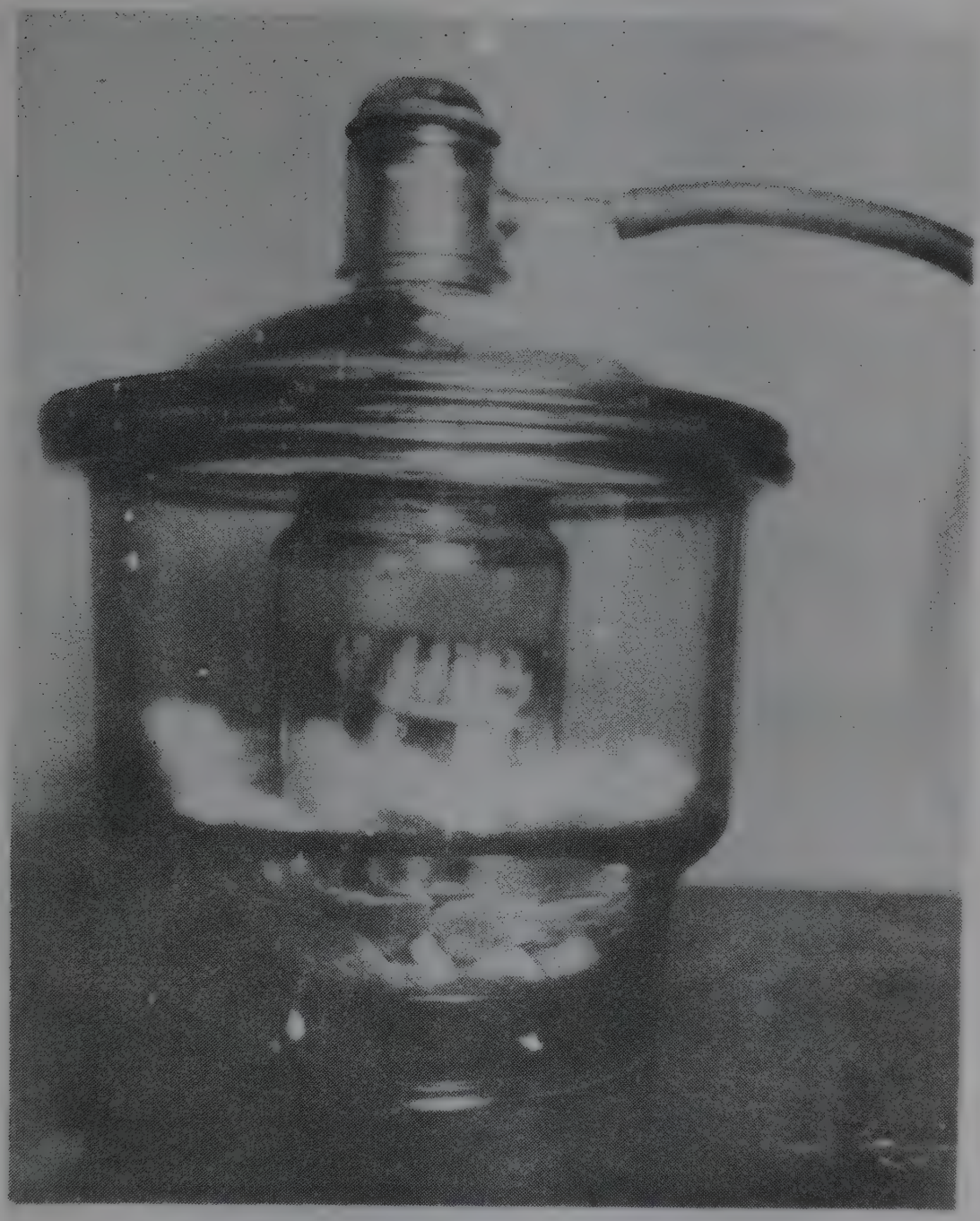

FIG. IV–A.

DNA - Methyl green substrate

During preparation of this substrate for ADNB titration, a few drops of choloroform were added as preservative. The substrate as such was stored at 4°C and used up within 2 to 4 weeks. If contamination was detected by development of turbidity or mold growth, the substrate was discarded and a fresh batch prepared. For long term storage, aliquotes of the substrate were lyophilized.

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